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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

July 1, 1974 through June 30, 1975

Part III A

DIVISION OF CANCER CAUSE AND PREVENTION

U.S. NATIONAL CANCER INSTITUTE
Division of Cancer Cause and Prevention
ANNUAL REPORT

July 1, 1974 through June 30, 1975

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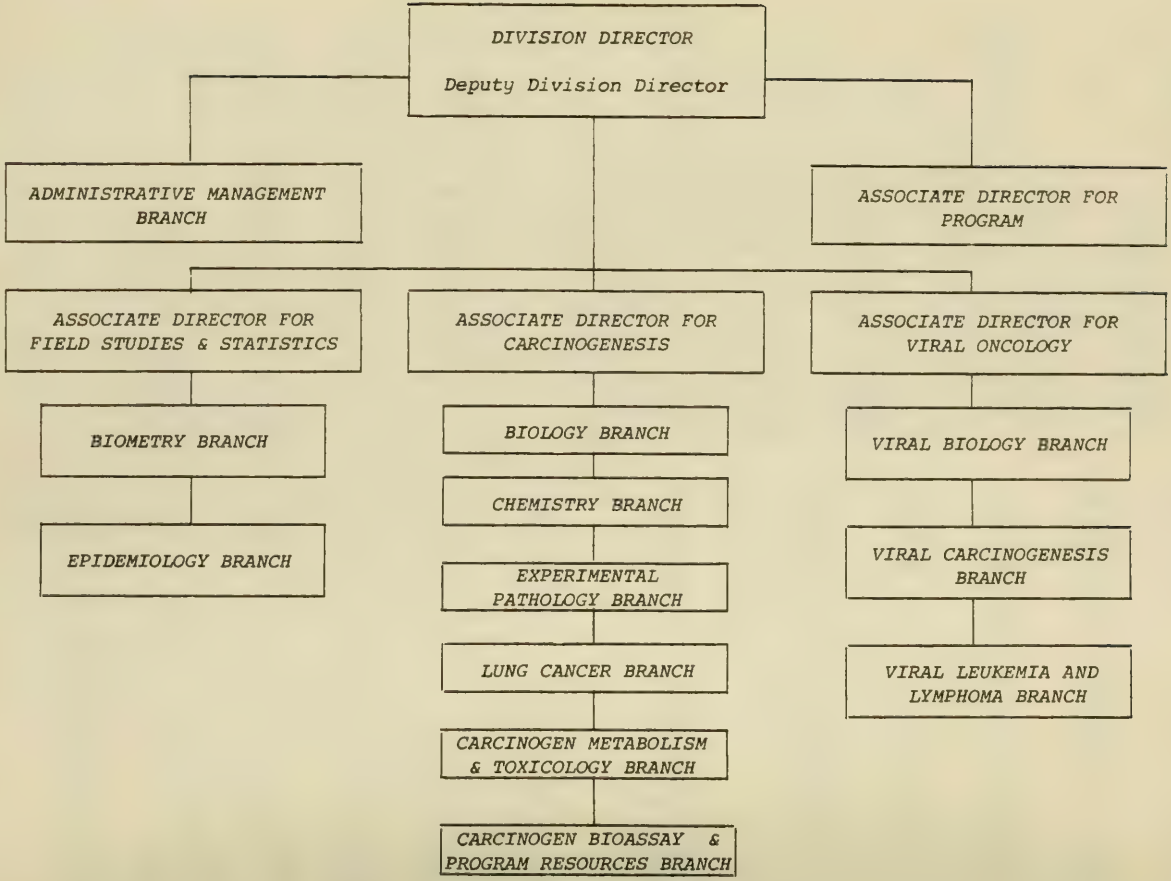
DCCP Organizational Chart



SUMMARY REPORT
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE
July 1, 1974 - June 30, 1975

The summary report of the Director, Division of Cancer Cause and Prevention, sets forth goals and objectives of the Division, a conceptual base upon which a broad attack on cancer etiology and prevention can be developed, objectives and program approaches to be used for their attainment, and the magnitude of the task and investments required.

A simplified synopsis of major goals, and of pathways to them, is given in Figure 1. More detailed program logic networks for Field Studies and Statistics, Carcinogenesis and Viral Oncology will be found under specific area presentations.

Activities in DCCP are specifically directed towards cancer prevention. Research output is continually utilized in resetting more advanced priorities and in formulating those activities that offer the highest promise of success. The major structures of this scientific management process are indicated in Figure 2.

Newly enacted legislation and funding for the National Cancer Program pose novel challenges and opportunities in research and for cancer control activities in man. This legislation and program recognize the promising advances of cancer research which now make it possible to formulate direct intervention in man's behalf: the definition and engineering of a less hazardous environment and the identification of susceptible individuals.

Scientific, public and legislative awareness in the role of environmental factors as cancer causes has increased considerably during the last year. This is particularly reflected in the current interest of the National Cancer Advisory Board and its subcommittee on environmental carcinogenesis in the formulation of expanded activities in this field.

IMPACT OF CANCER

Cancer occurs in all parts of the world, but variation in the incidence of specific forms of cancer is the rule rather than the exception. A range of ten or twenty-fold is common and for some types of cancer it is far wider.

The mean rate of cancer mortality in the United States occupies an intermediate rank in the worldwide range, but the rank of mortality from specific types of cancer varies markedly. The United States' white polulation has the lowest mortality from cancer of the stomach and close to the highest from cancers of the colon and female breast. As shown in Table 1, the incidence rates of various cancers in the U. S. are expected to increase from a low of 25% each for leukemias and breast cancer to a possible high of 38% for cancer of the pancreas. This table also presents leads towards identification of etiologic factors and towards prevention.

Available evidence suggests that environmental agents and social practices, rather than conventional genetic factors, are largely responsible for variation in the occurrence of cancer in different populations. It should therefore be possible to reduce the occurrence of the major types of cancer in the United States to the level of the lowest ranking country for that type. Such a reduction would cut mortality from cancer by one-third. This is the minimum goal we should aim for.

More than 52 million Americans now living will eventually have cancer (one in four persons) according to present rates. Cancer will strike over the years in approximately two of three families. In the 1970's in the United States alone, there will be an estimated 3.5 million cancer deaths, 6.5 million new cancer cases, and 10.0 million under medical care for cancer unless the present trends are moderated. Such trends mean that in 1976 there will be 346,000 cancer deaths and 747,000 new cases and in 2000, 471,000 cancer deaths and 1,085,000 new cases.

Table 1 also indicates the overwhelming causative effects of cigarette smoking and dietary habits: Perhaps as much as two thirds of all cancers are related to these factors. Rapid progress is likely to take place in the control of smoking-related cancers through educational campaigns, the development of less hazardous cigarettes and the identification of individuals at risk. Dietary factors appear more complex and clear cut solutions will need to wait until more fundamental research and questions are resolved. Occupational carcinogens are also being detected with increasing success; although it is estimated that less than 10% of all cancers may be of occupational origin, the identification of occupational risks usually leads to rapid and effective preventive measures.

Although cure rates for cancer patients have steadily improved over the past several years, the mortality (both in actual numbers and in rates corrected for population growth and other changes) continues to rise. This is because new cancer cases are appearing at an even greater rate than the improved rate of cures.

PROGRESS, PROBLEMS AND OPPORTUNITIES

A. FIELD STUDIES AND STATISTICS. Cancer can be prevented by identifying its causes and removing them, and/or by increasing the body's defenses against them. Thus, it is important to know not only what causes cancer, but also who is unduly susceptible or resistant. Susceptibility may be immense. For example, if an identical twin develops leukemia during the first year of life, his co-twin is virtually certain to develop leukemia within weeks or months. This increased risk fades quickly with age, and entirely disappears by 6 years of age.

Recently there has been a substantial increase in our list of characteristics of persons who are unusually susceptible to leukemia, lymphoma and other cancers. Once such a list has been created, it can be examined for a common denominator. Many items can be explained by a known or possible genetic defect, sometimes extensive enough to cause various abnormalities in the appearance of chromosomes. It should be noted that the only environmental agent known to be leukemogenic in man, ionizing radiation, and the strongest suspect among the chemicals, benzene, produce long-lasting chromosomal abnormalities in somatic cells.

By contrast, persons at high risk of lymphoma (solid tumors of lymph tissue) have inborn immunological deficiencies (ataxia-telangiectasia, Wiskott-Aldrich syndrome, or congenital x-linked agammaglobulinemia). Consistent with these observations is the increase reported in the occurrence of lymphomas among persons given immuno-suppressive drugs following renal transplantation. A sudden upturn in one form of lymphoma, Hodgkin's disease, at about 8 years of age, also suggests a relationship to immunological development. Normally, at this age, there is a marked spontaneous involution of lymphoid tissues throughout the child's body. Lymphoid tissues are centers of immunologic activity. It is tempting to think that some failure in this normal regression accounts for the upturn in the occurrence of the neoplasm.

There are many other inborn defects that carry high risk of cancer of various types. In the aggregate, they do not account for a large proportion of cancer but they probably do constitute a large percentage of cancers among young people. In this group, prevention or early detection and treatment can provide decades of healthy life. Peaks soon after birth in the frequency of diagnosis of leukemia, neuroblastoma, Wilms' tumor, and certain forms of brain cancer indicate that many of these neoplasms are likely to have been initiated during intra-uterine life.

Knowledge of these relationships permits physicians to examine children with certain defects more closely than usual, since early detection and prompt removal of these tumors can be life-saving. Scientists, on the other hand, can explore the origins of the tumors in the light of what is known of the associated birth defects. Thus, instead of studying a tumor in terms only of itself, new avenues of approach have been opened. What is learned through these rare mistakes of nature may well apply to prevention of large categories of cancer. In particular, the epidemiologic identification of individuals and populations at increased risk of different cancer patterns aids the investigator in virology and chemical carcinogenesis in searching for specific etiological factors.

International Activities

A contract with NAS-NRC supports and extends cancer research at the Atomic Bomb Casualty Commission in Japan. This has paid off in a modified Japanese attitude toward ABCC, so that there is now substantial involvement of several Japanese universities, including the development of a tissue registry at Hiroshima. These resources provide on-the-ground data resources for the development of epidemiologists. The Japanese Migrant Studies in Hawaii (in cooperation with the Heart and Lung Institute) have provided a very strong suggestion that meat - in particular, beef - is associated with the great increase (4-fold) in cancer of the large bowel in Hawaiian Japanese. This work is being followed up by work in the Carcinogenesis Area in the search for bacterial flora possibly capable of producing carcinogens in the lower bowel.

The field studies with the International Agency for Research on Cancer (IARC) in Iran on cancer of the esophagus have progressed very well.

Under the aegis of the UICC, the Epidemiology Branch is collecting data for an international comparison of pediatric cancer around the world. A monthly newsletter is used to communicate important observations on the origins of childhood cancer to pediatric oncologists throughout the world.

The international registry of epidemiology and epidemiologists (as a part of the International Cancer Data Bank) is about to begin operation as a cooperative activity of UICC and NCI.

Work has continued on exploiting special populations in India (the Bombay Cancer registry) and in Cali, Colombia (problems of cervical cancer, Hodgkin's Disease). Migrant studies of Norwegian and Polish migrants to the United States are being continued - although at a relatively low financial level.

The cooperative work with Soviet Russia in cancer epidemiology was confirmed at a conference in Washington during February 1975. U. S. data on incidence, mortality and survival, plus some special data on skin cancer have been sent to Soviet Russia.

Domestic Activities

Two major thrusts define domestic studies: data gathering and analysis, and the testing of etiologic hypotheses. With respect to the former, the report of the Third National Cancer Survey (TNCS) was published, for all three years of the survey, and by survey area. Also cancer mortality data, by county, by site, by sex and race for the 20-year period 1950-1969 were published, giving total numbers of cases, and rates. This will be followed by maps, permitting visual identification of clusters. Hospital cost data have been gathered by the TNCS. (The median hospital payments made within two years of diagnosis are between $2000 to $3000). Information on treatments and survival has been reported in the 4th End Results Report. The Third National Cancer Survey and the End Results Program have given way to the SEER (Surveillance, Epidemiology and End Results) Program. Through this program, we hope to develop early data on incidence, mortality and survival, on a grass-roots level, hopefully to provide contemporary data to guide the search for etiologic factors and other control activities.

A special interest in familial and genetic cancers and in childhood cancers continues. Familial cancers (e.g., medullary carcinoma of the thyroid and pheochromocytoma) give an opportunity for effective secondary prevention through frequent examination of family members at high risk. Childhood cancers are being pursued through a national registry of death certificates, a followup of children born in military hospitals where data exist on their intra-uterine exposure to x-irradiation, and in cooperation with the Third National Cancer Survey, will permit study of incidence as well as mortality.

The National Wilm's Tumor Survey has confirmed the high rates of aniridia, and hemihypertrophy in Wilm's tumor children. These are in addition to previously observed high frequency of genito-urinary defects and familial incidence.

Other major areas of work involve studies of prognostic factors, particularly in breast cancer, and Hodgkin's Disease. An emphasis on the characteristics of patients who may or may not benefit from certain forms of therapies was developed out of the cooperative studies of cancer of the prostate, undertaken with the Veterans Administration. These studies helped tailor treatments to prostate cancer patients. The concept of the "Epidemiology of Treatment" arose out of these studies and it should develop into a fruitful new field of study.

B. CARCINOGENESIS. The observed relation between exposure to certain chemicals and the development of cancer in man has been historically the first step towards knowledge of the etiology of cancer. A listing of agents presently known to produce cancers in man includes some 10 specific chemicals, 12 mixtures and crude products and, in addition, radioactive materials and radiation (including x-rays and ultraviolet light). The number of items is relatively small because studies capable of identifying an agent as directly causative of cancer in man require large-scale and time consuming epidemiologic studies and have been conducted for a very small proportion of the potential exposures of man. In fact, essentially all materials that have been demonstrated to be carcinogenic for man have also been found to be carcinogenic in animals.

Most chemical carcinogens known from animal studies appear active in a variety of animal species. It is estimated that more than 1000 chemicals have so far been found carcinogenic in animal bioassays out of about 6000 that have been tested.

The major types of cancer in man that can be directly related to known carcinogenic exposures are as follows: a] cancer of the lung related to cigarette smoke and certain other inhalation hazards (particularly asbestos, chromates, and radioactive materials); b] cancer of the skin related to exposure to a variety of crude tar products and combustion products as well as to ultraviolet light and other forms of radiation; c] cancer of the bladder related to exposure to aromatic amines and their derivatives; d] leukemias related to exposure to radioactive materials; and e] mesotheliomas related to asbestos exposure. A number of other human cancers have been considered to be related to exposure to environmental carcinogens but the evidence is less clear.

Because of the widespread use of some of these materials, the proportion of the total population exposed and affected is extremely difficult to estimate, with the exception of such materials as cyclamates, DDT and tobacco smoke. It has been estimated that the majority of the present cases of lung cancer in the United States are attributable to cigarette smoking and that factor alone appears to be responsible for a death toll in the neighborhood of 60,000 deaths a year.

The development of our knowledge on the causative factors of cancer in man can be projected along the following lines: a] epidemiologic studies to correlate specific exposures to certain types of cancer in man with particular emphasis on occupational exposures; b] additional bioassays in animal models which will identify chemicals as carcinogenic in animals and therefore potentially carcinogenic in man; c] studies of animal human correlations by compari-

sons of bioassay results with the results of epidemiologic studies or by bioassays on isolated human tissues by in vitro techniques; and d] by studies of pharmacologic and metabolic correlations among species.

The major areas of opportunity and need for progress in cancer prevention through studies in Carcinogenesis are: a] develop better means for the removal of hazardous chemicals from the environment; b] develop means to enhance the ability of the host to detoxify environmental agents; c] determine the extent to which chemicals act in synergistic concert with viruses or with physical carcinogens such as irradiation; and d] develop more rapid and more sensitive means to detect and bioassay the cancer-inducing effects of chemicals for man. The latter area of activity is particularly important. For example, it is estimated that approximately 200,000 new chemicals enter our environment each year. With present assays it takes approximately 2 years and 200 mice at approximately $200 per animal to test 1 chemical. It is hoped that newer studies in animal and human tissue cultures may diminish the 2 years and nearly $40,000 now required to test a single compound. However, until this is achieved the bioassay resources are limited to a few hundred compounds annually and it is necessary to select compounds to be tested on the basis of their distribution in the environment, intake in man and similarity to known carcinogens.

Progress Highlights

1] Bioassay. A contract at the Stanford Research Institute, directed at establishing a ranking of suspicion of carcinogenic hazard for compounds present in man's environment, provides information for the best utilization of current limited bioassay resources and may give early guidelines to the chemical industry, as to the hazards that may be expected by the introduction of a new compound.

The significance of current bioassays for carcinogenicity derives mainly from two considerations, namely, that, a] humans in general react like other animals to a variety of insults, the differences being usually quantitative rather than qualitative and, b] current bioassay methods, even when performed with adequate biometrical and qualitative controls, can be expected to detect only those compounds that are strongly carcinogenic.

These two reasons prescribe that the greatest attention be given to positive results in current carcinogenicity tests.

Testing and retesting of environmental chemical pollutants at NCI continue to include agricultural chemicals such as pesticides, artificial sweeteners such as cyclamates and saccharin, hormonal compounds generally used as oral contraceptives, and natural products of various molds and fungi. Food preservatives are also under constant scrutiny.

A bioassay prime contractor was selected to perform in a comprehensive way the many tasks involved in this area.

The establishment of standardized, detailed protocols for all the major bioassay procedures and for data acquisition and analyses has enabled the Program to make an important transition. Until now, the level of activity was mostly

aimed at the screening of environmental chemicals to identify suspected carcinogens for further study. The Program is now in a phase where more emphasis is given to generating detailed bioassay data at the level of sophistication and control necessary to meet regulatory requirements. Emphasis is placed on the need for documentation of all data entering into and resulting from a carcinogenesis bioassay.

Carcinogenic activity has been identified for several pesticides and pharmaceuticals. Several substituted hydrazines in widespread environmental use have been found to be markedly carcinogenic for a variety of organs.

2] Development of Bioassay Models. Major programs have been established for the development of biological models for tumor pathogenesis closely correlated to the main types of cancers in man. Lung Cancer and Colon Cancer Programs are well established; Pancreas Cancer and Prostate Cancer Programs, initiated in FY 1972, are now developing programs on Endocrine Cancers and childhood cancers. Their emphasis is on the use of animal models and human material to identify pathogenetic steps susceptible of inhibition or prevention.

In vitro models for neoplastic transformation of cells in culture by chemicals have been developed using cells from hamsters, mice, and guinea pigs. A major advance was the development of a host mediated transformation assay, responsive to carcinogens that require metabolic activation by the host. These new methods are being used to establish their value both as screening techniques and as tools for identifying key steps in carcinogen activation and interaction, susceptible to inhibition. The applicability of these techniques to transformation of human cells is currently being investigated. Genetic factors and growth conditioning factors required for neoplastic transformation by chemicals have been identified. Enhancement of chemical cell transformation by radiation and of viral transformation by chemicals have been demonstrated. Transformation of epithelial cells in vitro by chemicals has also been obtained in preliminary experiments. A major advance has been made with the transformation of human cells in vitro by chemical carcinogens, conditioned by corticosteroids.

3] Studies of Carcinogenesis Processes. New emphasis has been given to the study of carcinogen metabolism and toxicology, and a large collaborative effort is under way in the documentation of the conditions of formation and activation of nitrosamines. Pharmacologic inhibition of the formation of carcinogens has been obtained by blocking nitrosation of amines. Other classes of carcinogens are also under study (e.g., hydrazines, metals).

Major metabolic steps in the ensymatic activation of several classes of carcinogens have been identified with special emphasis on polynuclear hydrocarbons. Their reproducibility in human tissues has already been demonstrated in some cases. Micromethods, applicable to determination in small samples of human cells, have been developed for measuring carcinogen activation levels. Inhibitors of carcinogen activation have been identified. The development and use of high-pressure, liquid chromatography has been a milestone in the study of metabolism of polynuclear hydrocarbon carcinogens. This technique can separate as many as ten metabolites of benzo[α]pyrene in a short period of time and can therefore correlate carcinogen susceptibility with unique metabolic profiles.

4] Immunological Studies in Carcinogenesis. Radioimmunoassays have been developed for several carcinogens and related compounds. Progress was made in the study of the components of BCG responsible for tumor cell killing and in the study of the mechanism of action of cell-mediated immunity.

Refined methods have been developed for measurement of serum of carcinoembryonic antigen and rat alpha$_1$-fetoprotein.

C. VIRAL ONCOLOGY. A considerable number of viruses are known which either directly or indirectly cause tumors in different vertebrate species. Some of these replicate in and cause transformation of cultured human cells. The RNA viruses are responsible for many naturally occurring tumors in animals, and this group is most likely to be the cause of some human neoplasms. Certain DNA viruses may also be involved in oncogenesis either as causative agents or as necessary co-factors in cell transformation. Thus far, the only DNA viruses associated with natural cancers of animals and man are herpesviruses. Although candidate viruses have been isolated more frequently in recent years, none as yet has met the rigorous criteria to be considered a human tumor virus. Even if virus particles may not be the direct cause of human cancer, virologists are pursuing the possibility that cellular genes - genes containing information similar to that of RNA or DNA tumor viruses - are expressed in human cancer.

Current knowledge suggests several methods for analysis of human tumors for virus-specific genetic information: 1] Molecular hybridization between cancer cell RNA and natural synthetic DNA prepared by viral RNA-DNA polymerase; 2] Search for RNA-DNA polymerase activity in human cancers; 3] Search for human virus-specific antigens related to known tumor virus antigens in human cancers; 4] Application of new techniques (e.g., chemical treatment) for the induction of virus or virus genetic information in human tumor cells.

1] RNA Viruses:

Characteristics. RNA tumor viruses possess a number of similar biological and biochemical characteristics, are widely distributed in vertebrate species, and are transmitted vertically under natural circumstances. Infections are often covert, resulting in only partial expression of the viral genome. Each type C virus, regardless of origin, possesses a major internal antigen which permits species delineation (gs-1), and shares a determinant which is interspecies specific (gs-3). There is now good evidence that internal antigens can be detected in embryonic, adult, and tumor tissues of various animal species. This finding is highly significant because the antigen can be found in situations where no virus can be demonstrated.

Of greater importance in terms of understanding the determinants of cancer is the reported linkage between RNA tumor virus and early viral gs antigen expression with the development of cancer late in life.

All RNA tumor viruses contain 60-70S RNA and enzyme known as RNA-dependent DNA polymerase (RDDP) which permits transcription of viral RNA into DNA. As an antigen, RDDP, like the gs antigen, has both species and interspecies characteristics.

To provide a basis for further understanding information was sought to elucidate the mechanisms involved in the integration of RNA tumor virus information into host cells, and the relationship of this reaction to cell transformation.

Within the past year progress has been made toward the understanding of the biochemical (molecular) events which occur in the process of cell transformation. These studies are important because they can be applied to human normal and tumor cells to identify virus-specific sequences that have characteristics similar to those of animal RNA tumor viruses. Such studies also help to provide information on mechanisms of virus expression and regulation which could lead to new methods for controlling human cancer at the cellular level.

Analysis of human cancers for evidence of RNA tumor viruses. The powerful tools of molecular hybridization have permitted the analysis of human tumors for viral genetic information. Evidence from animal model systems indicate thus far that every cell line infected with or transformed by RNA tumor viruses possesses virus-specific RNA sequences readily detectable by molecular hybridization. Corresponding neoplasias of murine and human origin were found to exhibit remarkable similarities. For example, human breast carcinomas contained RNA possessing sequence homology to that of murine mammary tumor virus (MMTV).

Following reports of RDDP in the virions of RNA tumor viruses, it was important to determine whether the enzyme is restricted to tumor viruses and whether it is restricted to tumor cells. Attempts are now being made to determine whether the polymerase is present in human cells. RDDP obtained in the particulate fraction (1.16 g/m) of human leukemic cells was purified and characterized. The enzyme could be distinguished from major DNA polymerases of normal cells.

Considerable effort is being directed to search for RNA viruses which might be oncogenic for man. For the past several years sufficient numbers of observations reporting the finding of particles similar to Type C and Type B viruses in human malignancies have been made to conclude that viruses of these types may also infect man.

Attempts to confirm earlier findings showing a correlation between the presence of virus-like particulates in human milk, the RDDP activity associated with fractions separated from the milk, and the breast cancer history of the milk donor have not been successful.

2] DNA Viruses:

Characteristics. As recently as 1964, herpesviruses were generally considered to lack oncogenic activity. Prior to that time, the only evidence linking these viruses to animal neoplasias was the finding of herpes-like particles in cells of renal adenocarcinomas of the frog. The discovery of the Epstein-Barr virus (EBV) in cultured lymphoblastoid cells from patients with Burkitt's Lymphoma in 1964 stimulated extensive studies to examine herpesviruses as possible etiological agents of cancer in several animal species, including man.

Herpesviruses interact with their host cells in a productive or non-productive manner. During the productive growth cycle, the synthesis of infectious progeny is invariably accompanied by destruction of specific target cells. The non-productive cycle is very similar to that observed with other oncogenic DNA viruses. Stimulation of cellular DNA synthesis, acquisition of virus-induced antigens, incorporation of viral nucleic acid and transformation of normal cells into established lines capable of indefinite proliferation have all been described. Activation of virus synthesis in non-productively infected cells by exposure to mutagens (BUDR, IUDR) or irradiation is usually paralleled by cell death. Herpesviruses, like the RNA tumor viruses, also establish persistent covert infections. Whether latent herpes infections are the result of low-level productive or non-productive interactions has not been determined.

The mechanisms underlying herpes-induced oncogenesis remain obscure. The close association of these viruses with several animal malignancies suggests, but does not prove, their involvement as etiologic agents.

Relationship to human cancer. The most outstanding evidence for the existence of human oncogenic herpesviruses is derived from seroepidemiological, biochemical and biological studies, suggesting an association between various herpesviruses and specific malignant diseases of man. The Epstein-Barr virus is suspect of playing some role in the genesis of Burkitt's Lymphoma, nasopharyngeal carcinoma and, to a lesser extent, the sarcomatous form of Hodgkin's Disease and chronic lymphocytic leukemia.

The Epstein-Barr virus is closely associated with Burkitt's Lymphoma (BL) but the absence of an experimental animal susceptible to infection with this agent has made it difficult to pinpoint its role in the induction of this disease. Therefore, although the evidence which suggests that EBV is the etiological agent of Burkitt's Lymphoma is circumstantial at present, it is so strong that a causal rather than a casual relationship is suggested. The tumor is most prevalent in regions of Africa where conditions are extremely favorable for insect-vectored disease. Cases in certain areas have been shown to cluster in time and space.

A serological association has also been described between EBV and nasopharyngeal carcinoma (NPC) that is as pronounced and consistent as that observed in Burkitt's Lymphoma.

The relationship between EBV and Hodgkin's Disease (HD) is not as clear as that observed between EBV and BL or NPC. Clustering studies strongly suggest a horizontally transmissible agent as one factor in the pathogenesis of the disease. Although increased antibody levels to EBV have been recorded in persons with HD, it is clear that a significant percentage of patients have no detectable titers.

Although these arguments are highly suggestive, they are not conclusive as far as the etiology of BL and NPC are concerned. While there is good reason to believe that EBV is involved in the oncogenic process, its role could be that of an accessory factor or passenger virus in the tumor. No EBV DNA has yet been found in American BL tissues by the RNA-DNA hybridization technique, even though this tumor is histopathologically similar to African BL. RNA sequences

homologous to that of the Rauscher murine leukemia virus RNA have recently been described in BL biopsies suggesting the possible interaction of Type C viruses in the genesis of this disease. If EBV acts to prime the cell for neoplastic change, it is possible that one or more combinations of environmental and host factors interact to promote oncogenesis. Thus, the association of EBV infection with more than one disease becomes more plausible.

3] Treatment and Control.

A rational approach to tumor therapy may not depend on the isolation of a bonafide human tumor virus. Indeed, existing model systems have provided sufficient information which can be applied to the control of human cancers. For example, chemical inhibitors (antienzymes, gene repressors) which act on specific stages of viral replication or stimulation or host immune mechanisms (vaccines) to virus or virus-mediated, tumor-specific antigens may prevent or control oncogenesis.

Formalin killed vaccines prepared from murine leukemia viruses have produced significant reductions in tumor incidences in mice given potent chemical carcinogens. Because of the reported immunological and biochemical relatedness between MuLV (Rauscher) and human leukemic cells, terminal cancer patients were immunized with inactivated RLV. Evidence for humoral and/or cell-mediated immunity against this virus was found in more than one-half of the immunized patients. Whether this treatment produces beneficial effects for the patients can be only determined after many years of observation.

SUMMARY

Between 30% to 90% of cancer deaths in man is preventable. Mortality statistics from around the world show that if cancer mortality in the United States, site for site was comparable with that of the lowest country reporting for each site, approximately 100,000 lives would be saved each year. The major sites in which gains should be made are cancer of the colon and the rectum, cancer of the lung, breast cancer, cervix and uterus (particularly among non-whites, cancer of the ovary, cancer of the prostate, and probably leukemia.

Information is already in hand to reach these goals for lung cancer and for cancer of the cervix and uterus. These 2 sites alone would account for 100,000 or more savable lives annually. A concerted research effort is called for in cancer of the lung, colon, breast cancer and cancer of the prostate. There is promise of substantial pay-off in these sites. For example, migrant studies have provided very strong evidence that cancer of the colon is a disease with a very strong environmental base, possible related, among other, to high beef meat consumption. Japanese migrants to Hawaii show a 3 to 5-fold increase in contrast to Japanese on the home islands. When we find all elements of the new environment that lead to so large increases, application of this knowledge to the present United States population could reduce the U. S. incidence by another 25-30,000 lives a year.

Thirty percent (or 100,000 lives) is a minimum estimate. If we add to the preventable cases (in terms of current knowledge, or knowledge that should be available in the next few years, earlier diagnosis and improved treatments --

where prevention has broken down), this number should be easily doubled. For example, if the most recent knowledge on early diagnosis of breast cancer were applied across the country, we might anticipate 12,000 fewer deaths each year from breast cancer alone.

Current work on identifying the cancer "susceptibles", if successful, will lead to cutting down cancer deaths. First, by identifying susceptibles, we will know what people to watch closely and regularly, so that we might diagnose their disease early, and thus treat it successfully, Second, by identifying the cancer susceptibles, it should be possible to protect them from those environmental assaults which could be carcinogenic to them - while essentially innocuous to others.

Ninety percent reduction in cancer deaths could be reached within the present century, if, in addition to current knowledge on prevention, and universal application of the best diagnosis and treatment techniques, our research uncovered, a] the interacting environmental elements that cause migrants' cancer rates to change toward those of their new countries; b] those environmental elements which lead to different rates in different countries in the world; and, c] the susceptibles whom we must and can protect.

While there are major problems in the prevention of virus-induced cancers, not heretofore experienced in other areas of infectious disease, we should anticipate substantial inevitable success in the control (including prevention) of these diseases in man. These problems include the probable difficulties in using conventional vaccines for prevention if a major means of virus exposure is vertical rather than horizontal.

These difficulties notwithstanding, opportunities for cancer prevention and control are very bright through application of existing knowledge and through a prioritied commitment to seek new information with innovative application for man.

The most promising areas now developed are in the control of cigarette smoking related cancers, occupational hazards and, to a less precise extent, in diet-related cancers.

A Note on Management. The obligatory opportunity to plan and manage biomedical activities from basic or fundamental research upwards through development and application, with effectiveness and efficiency, requires skillful staff and line personnel of unabashed motivation, objectivity, and tact. Tact is of paramount importance because NCI management must persuade constituent scientists to attack specific problems of human disease. We adhere to the belief that NIH exists primarily to allow people to live better and longer and that NCI abets this goal through the conduct of research and development for prevention and treatment of cancer in man. Tact, including the courage and reason to say no, is important also because while the need for widespread program participation is high, many scientists do not understand that even successful completion of their proposed project may not necessarily contribute to the attainment of program goals. This thrust towards the timely control of disease must be tempered with appreciation for and selection of fundamental projects with some expectation of contribution to eventual application to man. This can and should be done through both grants and contracts.

We are fortunate to have this admixture of talents and motivation in the Deputy Director, Dr. G. B. Gori, and Associate Directors, Drs. Moloney, Saffiotti and Schneiderman; the Assistant Director, Dr. W. G. Flamm; and the Assistant to the Director, Dr. R. Depue; and in the Administrative Officers, Mr. John Miller and Mr. Nicholas Olimpio. Particular recognition is entirely warranted to their associates, whose motivation to program goals exceeds their desire for personal recognition.

Please refer to summary and individual reports for details of activities within DCCP Programs for FY 1975.

CANCER ETIOLOGY AND PREVENTION PROGRAM

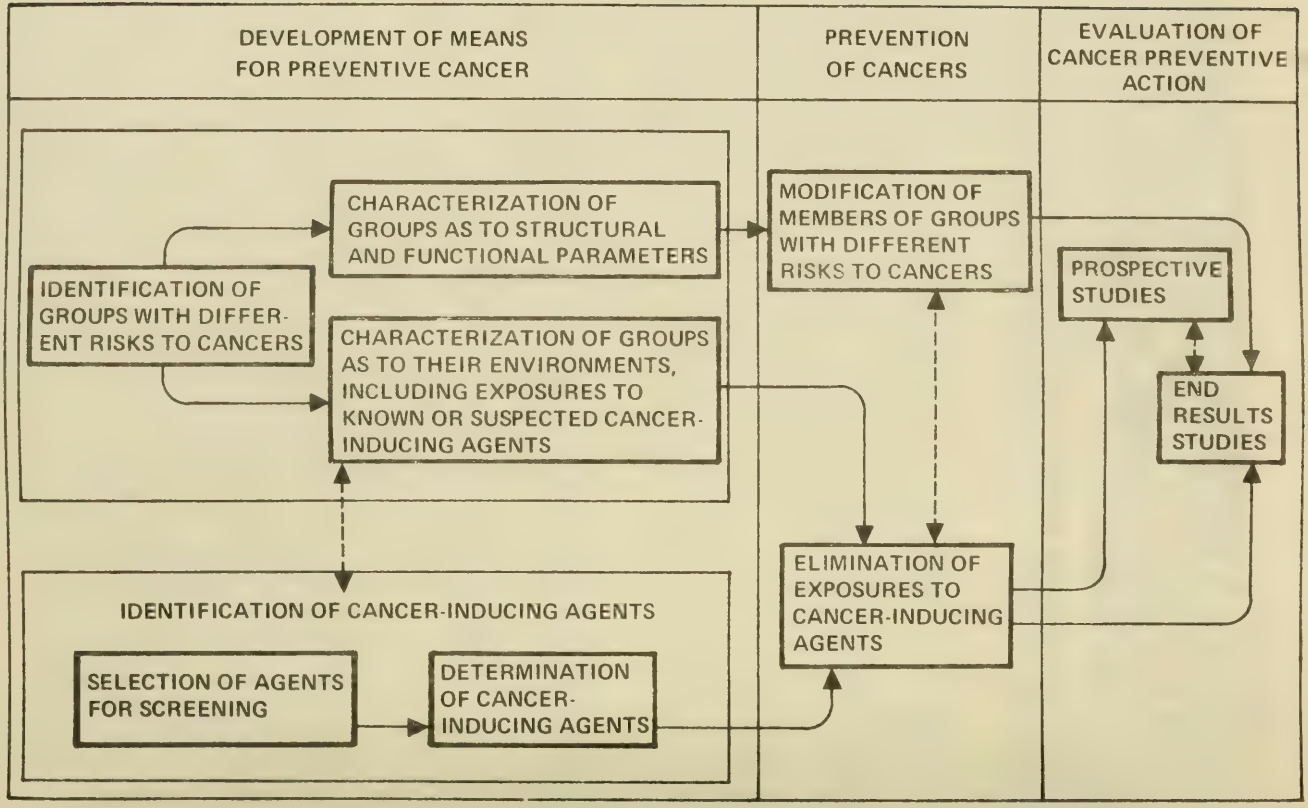

DEVELOPMENT OF MEANS FOR PREVENTIVE CANCER
PREVENTION OF CANCERS
EVALUATION OF CANCER PREVENTIVE ACTION
IDENTIFICATION OF GROUPS WITH DIFFERENT RISKS TO CANCERS
CHARACTERIZATION OF GROUPS AS TO STRUCTURAL AND FUNCTIONAL PARAMETERS
CHARACTERIZATION OF GROUPS AS TO THEIR ENVIRONMENTS, INCLUDING EXPOSURES TO KNOWN OR SUSPECTED CANCER-INDUCING AGENTS
IDENTIFICATION OF CANCER-INDUCING AGENTS
SELECTION OF AGENTS FOR SCREENING
DETERMINATION OF CANCER-INDUCING AGENTS
MODIFICATION OF MEMBERS OF GROUPS WITH DIFFERENT RISKS TO CANCERS
ELIMINATION OF EXPOSURES TO CANCER-INDUCING AGENTS
PROSPECTIVE STUDIES
END RESULTS STUDIES

FIGURE 1

FIGURE 2

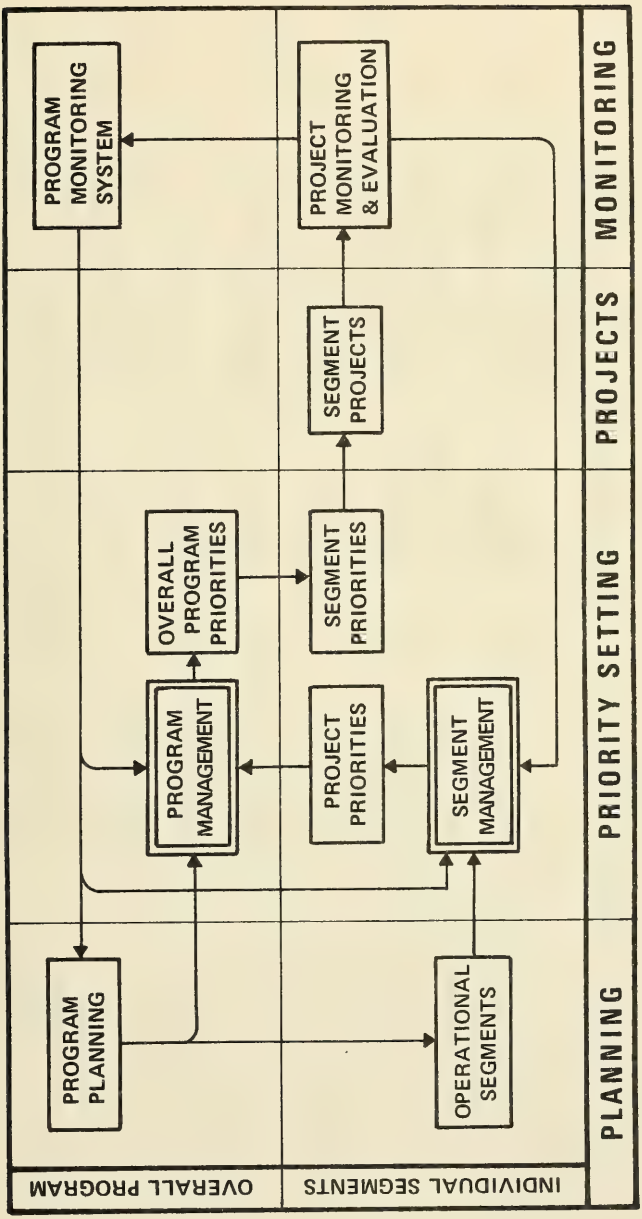

PROGRAM MONITORING SYSTEM
PROJECT MONITORING & EVALUATION
SEGMENT PROJECTS
OVERALL PROGRAM PRIORITIES
SEGMENT PRIORITIES
PROGRAM MANAGEMENT
PROJECT PRIORITIES
SEGMENT MANAGEMENT
PROGRAM PLANNING
OPERATIONAL SEGMENTS
OVERALL PROGRAM
INDIVIDUAL SEGMENTS
PLANNING
PRIORITY SETTING
PROJECTS
MONITORING

TABLE 1: CANCER INCIDENCE: EXPECTED NUMBERS, 1970-2000; CHANGE IN RATES, 1970-2000; DEATHS, 1970

Site	New Cases (Incidence)* 1970	2000	Deaths	Incidence Year 2000 % Change in Rate**	Major Causation	Means of Prevention
Lung	81,000	295,000	62,000	179	Tobacco Smoke Air Pollution (including on-the-job)	Stop smoking - Reduce pollution Less hazardous cigarette
Large and Small Bowel	92,000	134,000	44,000	11	Intestinal Flora? Heredity? Diet?	Viruses? Other insults. Identify susceptibles and eliminate their exposure.
Breast	82,000	111,000	30,000	4	Virus? Diet? (Hormones) Genetic?	Vaccines. Identify susceptibles.
Pancreas	20,000	35,000	18,000	38	Diet? Virus? Other Insults?	Identify etiology. Identify susceptibles.
Prostate	51,000	78,000	17,000	17	Hormones? Diet?	Identify etiology. Identify susceptibles.
Stomach	21,000	4,500	16,000	-84	Diet Poor socio-economic conditions	Diet modifications. Sociologic modifications?
Leukemias	20,000	32,000	15,000	26	Viruses. Radiation. Genetic.	Vaccines. Identify susceptibles and limit radiation.
Non-melanotic skin	376,000	585,000	5,000	20	Actinic rays. Genetic.	Limit radiation exposure. Identify susceptibles.
Misc.	242,000	408,000	75,000	30	Multiple	Identify extrinsic and intrinsic factors and modify them.

*Based on data from Third National Cancer Survey, 1969-1970. In situ cancers have been excluded.

**Projected change in age-adjusted incidence rates (year 2000 compared to 1970) assuming the trend in rates noted from 1947 to 1969 continues to the year 2000.

DCCP ANNUAL PROGRAM REVIEW DOCUMENT

FOR FY 1975

FREDERICK CANCER RESEARCH CENTER (FCRC)

The Frederick Cancer Research Center (FCRC) was established in the facilities at Fort Detrick formerly used by the Army for its biological research program. Litton Bionetics, Inc., was selected to manage and operate the Center beginning on June 26, 1972, with 12 separate research, development and resource projects, the major portion of these being extensions of Viral Oncology and Chemical Carcinogenesis programs in the Division of Cancer Cause and Prevention (DCCP). Also included in the initial projects were animal breeding, animal holding for Division of Cancer Biology and Diagnosis (DCBD), and biohazards control and the Advanced Systems Laboratory which was defined only in very general terms. These latter projects, with the exception of animal holding, were sponsored jointly by three divisions of NCI — DCCP, DCBD, and DCT (Division of Cancer Treatment). During the second and third years, the projects were extended to include the activation of a fermentation products pilot plant for DCT's drug development program, a special histopathology laboratory for DCBD, a chemical carcinogenesis safety program and support services for the Studies of Infectious Disorders of the Central Nervous System conducted by NINDS (National Institute of Neurological Diseases and Stroke). A new program, biologic markers research and analytical services, is being planned for DCT. While these new projects were being added, the projects in viral oncology and chemical carcinogenesis were being restructured into more cohesive programs to place more emphasis on research. In some instances, the expanded research has resulted from the transfer of NCI scientists with some of their staff to FCRC.

The most significant development during the current year is the addition of the Basic Research Program which was recommended by the Natonal Cancer Advisory Board as an implementation of the original Advanced Systems Laboratory. Dr. Michael G. Hanna, Jr., was selected as Director of Basic Research, and he, with Drs. James Ihle, Margaret Kripke and Isaiah Fidler, will develop a research porgram in the Immunobiology of Cancer. By the end of the third contract year, the four principal investigators will have been relocated to FCRC with little interruption to their respective research programs. At Frederick, they will be able to develop more effectively a research program having a central theme.

In the first three years of the operation of this Center, program planning including determination of priorities has been largely in the respective divisions and has reflected the needs within individual programs and the availability of funds. As useable space has become less available, the need for program planning has become more apparent. At the request of the Director of NCI, a group of NCI staff, assisted by three of the contractor's personnel, has recommended the development of a five-year plan to be revised annually, the strengthening of the NCI Advisory Committee for FCRC, and the creation of advisory and review committees. The implementation of these recommendations

should be effective in assuring the development of an outstanding program at FCRC.

For many of the programs established at the Center, major renovations and alterations have been necessary. Among these are the chemical carcinogenesis bioassay program in Building 539, viral oncology's viral carcinogenesis program in Building 522, viral oncology and chemical carcinogenesis research programs in Building 538, the Basic Research Program in Building 539, and animal holding in Building 571. At the Animal Farm, seven of the 24 breeding buildings have been extensively renovated and modernized, and the laboratory in the support services building has been remodeled. In addition, many smaller construction projects have been required because of differences in present and former programs, obsolescence or deterioration of facilities and systems resulting from age.

The overall responsibility at NCI for FCRC was assigned by the Director of NCI to the Director of DCCP although it is clearly recognized that the Center is an NCI-wide activity. The Project Officer and his staff of four are located organizationally within this Division while the Administrative Contracting Officer and assistant are assigned to Frederick by the NCI Research Contracts Branch. The Viral Oncology and the Chemical Carcinogenesis Programs of DCCP each have a scientific coordinator at FCRC to provide interface with NCI programs and performance evaluation for their respective areas.

VIRAL ONCOLOGY PROGRAM

The Viral Oncology Program (VOP) was established at the FCRC for the purpose of further implementing research in support of Viral Oncology and the NCI's program of coordinated research on viruses as etiologic agents of cancer. Program direction for the VOP is derived from the Associate Director for Viral Oncology with recommendations from the FCRC Viral Oncology Advisory Group (VOAG), consisting of seven NCI staff members including the FCRC Scientific Coordinator for Viral Oncology (SCVO) plus at least six outside consultants; program implementation is the responsibility of the SCVO who is located at Frederick. The major function of the FCRC VOAG is to assist the SCVO in reviewing all VO projects at FCRC on a continuing basis for the purpose of attaining and maintaining program excellence and to insure rapid response of the FCRC project operations to needs related to the overall NCI program objectives. The attainment of research objectives is further served through close associations between the FCRC SCVO, and the NCI Project Monitors, the Contractor's Project Managers, the Office of Program Resources and Logistics, the Office of Biohazards and Environmental Control, and other NCI program elements.

By the end of the third year, VO/FCRC work will have been performed within several projects, which were grouped into four main areas of operation at the start of the year. These include the (1) Production Area, which contained Project 1, Virus and Viral Reagent Production, for mass producing viruses and viral components to meet program needs; (2) the Research Area, which contained Project 2, Developmental Research, to adapt or create new techniques to grow viruses that are difficult to propagate, and Project 13, Basic Research in Viral Oncology, to conduct an NCI/Contractor collaborative program of basic research in direct support of existing VO programs pertaining to the role of viruses in

the etiology of human neoplasms; (3) Product Characterization and Standardization Area, designated as Project 3, to perform quality control tests on biological materials produced in Project 1; and (4) Biohazards and Environmental Control Area, to conduct a program of applied research related to the safe handling of hazardous biological materials. In addition to these work projects, VO partially supports FCRC effort in Project 4, Environmental Control, and Project 12, Animal Breeding. Finally, new this year, an Advanced Workshop was held at FCRC as part of the VO National and International Cancer Laboratory Liaison Program. Recognized national and international experts were invited to participate in a workshop-symposium, organized by VO/FCRC personnel in collaboration with the contractor, to address and recommend solutions for problems related to the production, concentration, and purification of Epstein-Barr virus and viral antigens.

The work scope of the FCRC VOP will be restructured next year; there will be a redirection of program effort (1) to take increasing advantage of project accomplishment in previous years and (2) to continue reflecting the need to further implement research in support of VO and the NCI's program of coordinated research on viruses as etiological agents of cancer.

The restructured VO/FCRC effort for the fourth year of operations will include the following: (1) DNA Virus Laboratory, (2) RNA Virus Laboratory, (3) Disease Control Laboratory, and (4) Virus/Reagent Production and Developmental Research Laboratory. It will be the function of these Laboratories to conduct basic or exploratory research on programs that are or could be vital to the Cancer Program. The research in the Laboratories will include some activities previously performed under Projects 1, 2, 3 and 13, and should take full advantage of unique opportunities at the FCRC to perform investigations for which a program need exists. In addition, the contractor will provide necessary facilities and support services for viral oncology investigators whose NCI operations are located at FCRC. Also as part of the FCRC VOP, there will be included the effort on applied research for Biohazards and Environmental Control, previously designated as Project 5.

It should be reemphasized that the FCRC VO effort is to be performed as a total integrated activity that will allow for a high degree of flexibility in response to overall program needs and for broad latitude in creating and pursuing fundamental research objectives both in-house and on a collaborative basis with outside acknowledged experts. As work in the given areas is performed, particular research objectives may receive changes in emphasis; objectives may be modified and/or previously established ones postponed or eliminated.

During the period 2 February 1974 to 19 January 1975, 11,695 liters of viruses were produced and 11,283 liters were concentrated, yielding a total of 16,511 ml of concentrated and purified virus. Of this amount, 12,521 ml were released to Program Investigators, thereby fulfilling 100 percent of that amount requested by the Office of Program Resources and Logistics for investigators. Seventy-three percent of the virus produced was Rauscher murine leukemia virus (RLV). Except for special 70S RNA preparations of RLV, the final products were generally characterized as containing 5 x 10^{11} virus particles/ml, 3,000 pico moles RDDP by endogenous assays and 300,000 pico moles RDDP by

exogenous assay, and 0.5 to 5.0 mg protein/ml. Woolly monkey virus (SSV-1) production accounted for 25 percent of the virus produced. Final products of this virus yielded 10^{10} particles/ml, RDDP activity of about 50 pico moles/ml/hr, and protein content of about 0.5 mg/ml. Two other viruses (SA4B and E25t) were produced in relatively small amounts.

In addition, a system for the large-scale production and purification of a RLV product containing intact 70S RNA was developed. The product is positive for 70S RNA by the simultaneous detection assay. Investigators evaluating the product have reported that the material is unnicked and denatures to about 60 percent 34S RNA.

Laboratory-scale studies on the production and purification of Gross leukemia virus and gibbon ape lymphoma virus were initiated.

A computer program, encompassing all aspects of large-scale virus production, virus purification, product characterization, and release of virus concentrates from the FCRC Virus Repository, was designed and tested. The program contains three master files on: production, purification and product characterization, and inventory control.

Two viral proteins (p30 and RDDP) have been purified from RLV by detergent lysis, followed by sequential ion-exchange column chromatography. They appear homogeneous by SDS-acrylamide gel electrophoresis. Their respective monospecific antisera have been prepared in rabbits and goats. These four viral diagnostic reagents are distributed to investigators at the direction of the Office of Program Resources and Logistics, NCI. A single 200 ml lot of concentrated RLV is processed each month. Since July 1974, a total of 4.865 mg of RDDP, 41.52 mg of p30, 16.50 mg anti-RDDP IgG, and 127 ml of goat p30 antiserum have been distributed to Program Investigators.

Studies were directed at defining culture methods for increased production of infectious EBV (I-EBV). The procedures were standardized as protocols and demonstrated I-EBV production at levels of 36 liters per lot with infectivity titers averaging 5.0 log 10/ml of 1000 x virus concentrate. Additional studies were directed at improving assay methods for I-EBV including optimization of test conditions and Raji cell growth and application of the above techniques to production assay and characterization of B-95-8 derived transforming EBV with regard to virus titers, specific EBV antigens, and virus morphology. An *in vitro* system for production of MMTV was developed based an dexamethasone stimulation of Mm5mt/c_1 mouse mammary tumor cells. This system was implemented into large-scale production with concomitant immunological (structural antigens), biochemical (viral reverse transcriptase), and biological (tumor induction) characterizations of the *in vitro* produced virus relative to mouse derived MMTV. Additional efforts in the electron microscopy laboratory resulted in development of improved techniques for virus particle resolution, including fixatives and buffers, and implementation of techniques for virus particle quantitation including the Sharp technique. Methods were developed for purification of RDDP, p30, and gp69/71, and RNase H from RLV. These techniques were applied to purification of these components from SSV-1. Monospecific antiserum was prepared against all of the purified components.

In studies with a candidate human virus, SA4B, cultures derived from a human liposarcoma were verified as being of human origin and not containing mycoplasma. They were shown to be carrying a virus which was released by induction techniques (IUDR and Dexamethasone). The parent cell was a low producer of this virus (STV); it was observed in thin sections of concentrated virus pellets but budding from cell surfaces was not detected. Uninduced cells contained an RDDP-like enzyme which was not neutralized by anti-murine RDDP IgG. The enzymatic activity was neutralized to 25 percent by anti-SSV-1 RDDP IgG. The viral enzyme was not characterized due to insufficient quantities of STV. Also, early hybridization results with STV preparations indicated that this agent was not related to mouse, but only to nonhuman primate SSV-1 virus. The products used in the hybridization procedures were characterized by equilibrium sedimentation ultracentrifugation analysis using cesium sulfate gradients. However, radioimmunoassays with 5000-fold concentrated STV indicated a strong cross to both murine p30 and p15 antigens. On the basis of these results, this candidate human oncornavirus program was terminated.

The Quality Control Unit has implemented procedures for carrying out exogenous and endogenous RDDP determination, simultaneous detection assays, total protein assays, complement fixation tests for RLV antigen, virus particle counts by negative staining and ultra-thin section with micrographic documentation, sterility testing for bacteriological, fungal and mycoplasmal entities, as well as in vivo and in vitro testing of RLV. Assay procedures have recently been implemented for determination of RDDP activity in MMTV preparations and for the titration of the early antigen of EBV.

A total of 3,738 samples were quality control tested during the January 1974-January 1975 interval. This level of effort, which was primarily in support of Project 1 program, represents a two-fold increase in the number of samples tested during the previous 12-month period.

Work associated with Basic Research in Viral Oncology was performed within seven subprojects. In the subproject, Virus Disease Modification, the effect of tilorones, levamisole and other immunostimulating drugs on ameliorating the course of disease induced in mice by RLV and LSTRA leukemia was studied. Tissue culture studies directed towards understanding the in vitro activity of tilorones, levamisole, staphylococcal membranes, and BCNU were continued. The effect of these various immunostimulatory compounds on the activity of the viral reverse transcriptase was investigated. The in vivo area of study continued on the immunosuppression caused by BCNU administration until November, at which time all work pertaining to this project ceased at FCRC.

In the subproject on Isolation and Characterization of Virus-Augmented Tumor Transplantation Antigens, a delayed hypersensitivity footpad assay has been developed to test for soluble tumor specific transplantation antigen (TSTA) induced by SV40. Hypertonic salt (3M HCl) solubilized TSTA gave a positive footpad reaction but did not protect against tumor cell challenge. In collaborative studies on transformation with DNA virus (HSV.2), the time sequence of antigen and antibody formation and cell mediated immunity were examined in a model murine system and salt soluble extracts from tumor cells are being prepared and tested for TSTA activity. Methodology has been

developed for explanting mouse mammary tumor virus-induced adenocarcinomas into tissue culture for other TSTA experiments.

Research in the Primate Virus Section focused on the role of human (Epstein-Barr) and nonhuman (Herpesvirus saimiri and Herpesvirus ateles) primate oncogenic viruses in neoplasia. Immunological studies have explored the association of Epstein-Barr virus with American Burkitt's lymphoma and nasopharyngeal carcinoma and the seroepidemiology of Hodgkin's disease. The purification and characterization of complement fixing, membrane and nuclear antigens from EBV and HVS cell systems, in addition to analysis of HVS structural proteins, has been initiated. A new DNA polymerase has been detected in EBV-producer cell lines. Studies in monkeys have emphasized the persistence of HVS and HVS-specific antibodies in experimental animals that do not develop malignant lymphoma and/or lymphocytic leukemia. The effect of heteropolymeric anion on enhancing DNA virus titers was investigated.

A murine cell line (238) was transformed following infection of the cells with UV-inactivated herpes simplex virus type 2. The transformed parent cell line (H238) produces tumors with a 100 percent incidence in fully immunocompetent BALB/c mice inoculated with 10^6 cells. Indirect immunofluorescence tests revealed the presence of HSV specific antigens in the transformed cells. HSV.2 specific antibodies, capable of neutralizing HSV.2 but not HSV.1 virus infections, were found in sera from mice bearing tumors induced by the transformed cells. The transformed cells do not harbor infectious HSV.2 particles, nor could infectious HSV DNA be rescued. In the same model system studies on the enhancement of transformation by herpes simplex virus (HSV) with certain chemical carcinogens were begun.

Laboratory renovations having recently been completed, endogenous xenotropic viruses are now being studied for their physiological role in the host, emphasizing the interrelationship of the host-graft response and steroid-gene interaction on control of expression of the viral genome.

Innovative utilization of the most recent electron optics instrumentation and techniques by the Developmental Electronmicroscopy Laboratory during the past year have resulted in several important contributions to the VO research program. A precise and reliable virus quantitation technique has been developed which eliminates the problem of operator fatique. A definitive study of morphological characteristics of human tumor cell lines has been submitted for publication and will provide the basis for study of these cell lines in their interactions with chemicals and viruses. A research program employing energy-dispersive X-ray analysis and immunochemical methods has been initiated.

Finally, experiments are being initiated to assess genetic and chemical factors related to the induction of endogenous type C RNA viruses and their role in the pathogenesis of murine leukemia.

In the National and International Cancer Laboratory Liaison program, laboratory facilities have been provided for long and short-term visiting foreign scientists. A highly successful international workshop on Epstein-Barr

virus production, concentration and purification was planned and held at FCRC in February 1975. Other activities included the writing of a laboratory manual on immunological methods useful in cancer research, and participating in the efforts of the International Agency for Research on Cancer and WHO/FAO programs to coordinate various efforts in oncogenic herpesvirus research.

In the Biohazards and Environmental Control effort, the chemical disinfection of RLV with alcohols, idophors and quaternary ammonium compounds has continued. Concentrations of 35% ethanol and 0.5% Wescodyne, are capable of inactivating 6.3×10^7 infectious units of purified virus in 30 and 10 seconds respectively; no detectable infectious virus was found post-treatment. The evaluation of a mass airflow animal containment system, using Newcastle disease virus in chickens and Sendai virus in mice, was successfully completed. Other sources of contamination were investigated and found to be only point sources of contamination. Eighteen cabinets have been received and evaluated for compliance to the NIH safety specifications. A variety of tests were performed to study the design and operation of Class II cabinets, including the use of fluorescein to determine filter integrity, the maximum biological challenge, lateral spread of a generated aerosol, personnel and product protection tests, and hazard assessment of biomedical equipment. A highly informative symposium for the annual safety information program was held at FCRC on March 19th on "Biohazards and Zoonotic Problems of Primate Procurement, Quarantine, and Research." Development of information data sheets has been initiated for incorporation into the "NCI Safety Standards for Research Involving Oncogenic Viruses."

As indicated above, the general scope of effort within which the Contractor will be required to conduct the VO/FCRC program in FY75 calls for the establishment of several laboratories. The DNA Virus Laboratory will be established for the purpose of determining the role of such viruses in the neoplastic process. Biological, serological, and biochemical techniques will be perfected and performed using specific purified viral components to search for evidence indicating the presence of these in human specimens and attempt to correlate experimental results with the incidence of human tumors. Research in the RNA Laboratory will be conducted to determine biological, biochemical, serological and other characteristics of type B and type C viruses under well defined and carefully controlled conditions. Comparative studies will be performed among viruses of these types to ascertain and identify properties that may either be shared or that are unique for a given virus. Information obtained will be used relative to further exploratory studies planned for new isolates, preferably of human origin. The purpose of the Disease Control Laboratory will be to pursue a multifaceted VO/FCRC program to study a variety of genetic, immunological, and viral synergistic factors that may influence the course of tumor development. The objective of these studies is to provide pertinent information as to the causative role of viruses in cancer and to develop rational approaches to the prevention and cure of these diseases. As this program proceeds, it is expected that collaborative working relationships not only with other VO/FCRC program elements, but also with NCI and contractor investigators who are engaged in studies of similar nature, will be established. Personnel and facilities in the Virus/Reagent Production and Developmental Research Laboratory will be utilized to perform all work related to the

large-scale production of viruses and viral components that are necessary for conducting laboratory research in the VO/FCRC or associated programs. This would also include control activities, to insure that products meet or exceed qualitative and quantitative standards. Plans have been made to produce a newly isolated human virus candidate and it is expected that work with this and possibly others, and cancer viruses from nonhuman primates (e.g. gibbon ape or woolly monkeys) will constitute a large portion of the effort in this Laboratory.

Effort in Biohazards and Environmental Control will continue to provide a biological safety research capability to elucidate biological and physical factors that may contribute to cancer risks associated with virus research and to develop control measures, including procedures and equipment, that will minimize the risks to man of laboratory acquired cancer and assure the protection of the general environment to the maximum extent practicable.

Finally the VO/FCRC program on the National and International Cancer Laboratory Liaison will proceed on an "as needed" basis with continued effort directed toward providing research facilities for visiting scientists, and participating in IARC, International Reference Center, and international symposia activities.

CARCINOGENESIS PROGRAM

Carcinogenesis research at the Frederick Cancer Research Center began when the laboratories were opened in June 1972. Four tasks were established at that time: (1) Selected Bacterial Species, (2) Large Scale Bioassay, (3) Preparation and Characterization of Carcinogens, and (4) In vitro Bioassay. The tasks had some common purposes and required a certain amount of collaborative effort, but they were not designed to function as part of a unified program. In subsequent contract renewals, the tasks became known as "projects," their functions were modified slightly and their titles changed accordingly to: (1) Laboratory Studies of Etiology of Cancer of the Digestive System, (2) Bioassay Research, (3) Preparation and Characterization of Carcinogens, and (4) In vitro Bioassay Methodology for Potential Carcinogens. In addition, two new projects were added: (1) Procedures for Safe Handling of Chemical Carcinogens, and (2) Chemical Carcinogenesis Research. During the present year, the Office of the Scientific Coordinator for Carcinogenesis, FCRC, has been the focus of a major planning effort which, through consultation with NCI staff and with advisors from other institutions, has led to the development of a plan for the future development of the FCRC carcinogenesis laboratories. Under this plan, the functions of the present laboratories and other new laboratories to be established in the future will be devoted to one central goal, the development of improved methodology for identification of human carcinogens and for the identification of population groups which are exposed to carcinogens. As resources become available for achieving this goal, a broad approach will be taken including research and development of improved techniques at the molecular, subcellular, cellular and whole animal level. At the present time, there is no laboratory in the world where all of these approaches to carcinogen identification are undertaken together, thus insuring comparison under controlled conditions.

Presently established laboratories have capabilities in the development of cell culture transformation tests for carcinogenesis, development of better animal bioassay systems, identification of carcinogens of endogenous origin related either to hormonal effects or bacterial metabolism, and analytical chemistry techniques and instrumentation to support these projects. In addition, a laboratory for the study of the critical target sites for carcinogen interaction in cells as an approach to carcinogen identification is in the beginning stages of development. This newest laboratory will require some expansion of personnel as well as provision of adequate laboratory space in order to make it an effective functioning unit in the mission of the FCRC Carcinogenesis Program. Other high priority areas for development include the following: (1) a considerable expansion of the presently very limited capability in organic synthesis in order to provide otherwise unavailable radioactively labeled carcinogens, postulated intermediates of carcinogen metabolism, and other reactive chemicals for study in the overall program; (2) development of a laboratory studying mutagenesis in various bacterial and eukaryotic systems as a technique for identifying potential carcinogens; and (3) the recruitment of personnel competent in studies on metabolic activation of carcinogens for the development of techniques suitable for metabolic activation of carcinogens in conjunction with in vitro test systems.

The present program organization is along the lines of the six projects listed above. It is likely that during the next year, a new plan of organization will be developed in collaboration with LBI's newly recruited FCRC Carcinogenesis Director, Dr. Charles Irving. The purpose of such a reorganization will be to more effectively accomplish the goals of the new program plan developed for FCRC.

The individual projects as they have existed during the past year and highlights of their research are listed below:

Laboratory Studies of Etiology of Cancer of the Digestive System — This project studies the role of materials in the digestive tract on the development of cancer within it. Particular emphasis is on dietary constituents and bile, their metabolism by intestinal tract bacteria, and the role of these materials and their metabolic products on the induction of cancer of the colon and rectum.

The Ames mutagenesis assay has been utilized in this project for screening tryptophan metabolites for mutagenicity and therefore for possible carcinogenicity. Except for a contaminant in one commercial sample, no tryptophan derivatives were found to be mutagenic nor was mutagenic activity found in the filtrates of cultures of intestinal tract bacteria cultured anaerobically with tryptophan.

Work on bile acid metabolism has included the development of improved methods for separation and identification of these materials. Nine of 13 human colonic bacterial species studied were able to hydrolyze the amide bond of the conjugated bile acid taurolithocholic acid under anaerobic conditions. At least two bacterial species (Bacteroides fragilis and Citrobacter sp.) were able to form the ethyl ester of lithocholic acid under anaerobic conditions when ethanol was present in the incubation mixture.

Bioassay Research — In this project, environmental chemicals are tested for carcinogenicity by administering them to rodents for a major portion of their life span. In addition, research is undertaken to develop improved techniques for the identification of environmental carcinogens. The goals of this research are to develop more reliable assay procedures, to find ways of reducing the cost of detecting carcinogens through bioassays without reducing their reliability, and to find more reliable ways to extrapolate results of these bioassays to the problem of carcinogenicity in man.

Research designed to improve the accuracy of estimation of MTD has included the development of a new computerized data system which continuously plots the weight of test animals compared to controls and solves for the dose which will produce a 10 percent weight depression. Twenty-one environmental chemicals are currently on chronic bioassay for carcinogenicity.

Preparation and Characterization of Carcinogens — This project functions as a resource for the synthesis, analysis, and distribution of chemicals of interest to the Carcinogenesis Program. Chemicals being tested for carcinogenicity are analyzed for their purity and stability under conditions of administration in bioassays. Carcinogens which are not commercially available and postulated intermediates in the process of carcinogens and noncarcinogenic analogs of known purity are maintained in a repository for distribution as chemical standards to other laboratories involved in carcinogenesis research.

In vitro Carcinogenesis — This project examines the methodologies of cell culture carcinogenesis techniques to determine conditions of maximum sensitivity and reproducibility, and to characterize end points to malignant transformation in vitro. A major goal is to standardize such techniques to make them widely useful in many laboratories for the screening or detection of environmental carcinogens.

A standardized hamster embryo cell in vitro carcinogenesis bioassay, using aliquot samples of cells stored in liquid nitrogen as the source of standard target and feeder cells, has been established. A list of standard chemicals with known carcinogenic or mutagenic activity was selected and is being used for evaluating the reliability of the system. To date, approximately 40 chemicals have been assayed. These include carcinogenic and noncarcinogenic analogs of some direct alkylating agents, polycyclic hydrocarbons, nitrosamines and amides, aromatic amines and aminozao dyes, and a few miscellaneous compounds. Almost complete correlation as to their reported carcinogenic activity in animal systems was found. A few procarcinogens such as diethylnitrosamine, N-2-acetylaminofluorene, auramine, and urethan, which require metabolic activation failed to induce transformed colonies.

Procedures for Safe Handling of Chemical Carcinogens — This project is being developed in order to help NCI formulate effective measures for the safe handling of chemical carcinogens in the laboratory and their subsequent disposal so that they will not be a hazard to either laboratory workers or to the surrounding community.

Chemical Carcinogenesis Research — Within this project, three areas of research are under development. These include: (1) immunologic studies on carcinogenesis and the identification of techniques for enhancing immunologic control of developing neoplasias, (2) studies on the interactions between carcinogens and cellular constituents required for neoplastic transformation and the utilization of this knowledge for identification of environmental carcinogens, and (3) investigation of the role of hormones in the induction of carcinogenesis and in determining the susceptibility of hormone responsive organs to carcinogenic chemicals.

Both estrogen and progesterone receptors have been demonstrated in the mammary epithelial cells of rats which were in a stage of development when tumors can be induced. Furthermore, the cytoplasmic-nuclear transfer of receptor appears to operate in the mammary gland.

Binding of DMBA to the DNA, RNA and protein of mammary epithelial cells was examined. As a control the binding to macromolecules of the liver, which is not susceptible to induction of tumors by DMBA, was measured. From these experiments it was found that the amount of DMBA bound to mammary epithelial DNA varies as a function of the age of the animal. There is a good correlation between the amount of DMBA bound and the incidence of tumors induced. Furthermore, in the 50-day old animal, the amount of DMBA bound to DNA increases as the dose of DMBA increases. The reported incidence of mammary adenocarcinoma correlates well with these findings. Binding of DMBA to liver DNA is much lower than that to DNA of the mammary epithelium.

There are two major problems which must be solved before the Carcinogenesis Program at FCRC will become fully effective as a major national resource for the development of improved and more economic test systems for carcinogen identification. First, although the staff initially recruited by the contractor has extensive training and experience in a number of areas of research, in-depth experience in chemical carcinogenesis was not present. Thus, there was a lack of guidance for these personnel in designing research of maximum effectiveness for solving problems of carcinogenesis and cancer prevention. Some steps have been taken to overcome this weakness during the past year. Dr. Charles Irving, a well recognized and respected scientist in the field of carcinogenesis, has been recruited to be Director of the Carcinogenesis Program for the contractor. He will join the staff toward the end of the present contract year. Dr. Douglas Janss was recruited from the National Cancer Institute to join the contractor's staff late in the last contract year. His experience in carcinogenesis of endocrine target organs has led to the establishment of a productive group working on this problem. Dr. Anthony Dipple, a highly qualified investigator of the critical sites of carcinogen interaction within the cell, has recently come from the Chester Beatty Research Institute in England to join the carcinogenesis staff. Other investigators of comparable qualification in carcinogenesis research will be needed to establish the groups working on mutagenesis and carcinogen metabolism as well as to strengthen the organic synthesis activities and some of the laboratories already in existence. The second major problem relates to the physical facilities available for mounting the type of program envisioned in the new plan for the FCRC Carcinogenesis Program. Although funds for the recruitment for the Carcinogenesis Director,

for Dr. Dipple, and for supporting personnel for both were included in negotiations of the present contract year which began on June 26, 1974, neither space nor the necessary renovation funds have been assigned for these groups. We cannot expect to retain such highly qualified individuals without very prompt attention to their space requirements. In addition, space will be needed in order to establish the other grups proposed above which are essential for a well rounded program on the development of better methods for carcinogen identification. By the nature of the new program, close consultation and collaboration among varied groups will result in a much more effective operation and thus more efficient utilization of NCI funds than will occur if the laboratory groups are scattered in different buildings resulting in less frequent communication. It would be highly desirable to plan to consolidate as many of these laboratories as possible into a single building.

ENVIRONMENTAL CONTROL AND SAFETY

The objective of this program is the control of the total work environment to minimize risk of human exposure to biological agents and chemical carcinogens and to guard against accidents involving radioactive materials, toxic chemicals or unsafe working conditions. An Employee Health Service has been established to assure that each employee is physically capable and will not be harmed in the performance of his work. Much of the effort has been devoted to developing safety protocols, testing of safety cabinets and other containment equipment and developing safe procedures for handling and storing carcinogenic chemicals.

This project works closely with the Biohazards Control Project in Viral Oncology and the Safe Handling of Chemical Carcinogens Project in the Chemical Carcinogenesis Area.

ANIMAL HOLDING AND ANIMAL BREEDING

More than 10,000 mice, rats, guinea pigs and rabbits are being held for DCBD for observation of effects following treatment at NCI or at FCRC and for naturally occurring morbidity and mortality. Approximately half of the animals have been moved from Building 539 at FCRC to the contractor's Falls Church, Virginia facility to provide space for the Basic Research Program's animals in Building 539. Upon completion of the renovations of Building 571 during the next year, the animals for DCBD will be moved back to FCRC and the number of animals in the program increased substantially.

The FCRC Animal Farm produces mice, rats, and guinea pigs for the programs at Frederick and other NCI programs. Twenty-one of the 24 breeding buildings will continue in operation so three will be available for renovation. Because of the need to increase the breeding of Strain 2 and Strain 13 guinea pigs, some special mouse hybrids and some strains produced in small numbers will be discontinued as will be the holding of animals until they reach the weight desired by some investigators.

SPECIAL HISTOPATHOLOGY

The NCI Histopathology Laboratory of DCBD has been located at FCRC to meet the need at NCI and to a limited extent at FCRC for special techniques that are not being met by the histopathology laboratories associated with the bioassay programs. Contractor's personnel are being trained by the NCI staff to take over an increasing amount of the workload.

SUPPORT FOR STUDIES OF INFECTIOUS DISORDERS OF THE CENTRAL NERVOUS SYSTEM

This laboratory conducts studies of slow, latent and temperate virus infections of the central nervous system in man and animals. The Contractor provides services in support of the NINDS research. When renovations being planned for Building 376 are completed within about a year, the program will be moved from its present location in Building 567 and the scope of work conducted by NINDS increased.

CHEMOTHERAPY FERMENTATION LABORATORY

Five of seven key scientific positions for this new research and fermentation program have been filled. The research staff was moved to the renovated laboratories in March from its temporary location. The research program has progressed in the isolation cultures, and screening of soil samples.

A computer-stored data bank for the rapid identification of microbial metabolites is being developed for the program.

The design of the fermentation plant was completed in March and completion of construction is planned for January 1, 1976.

OPERATIONAL PROBLEMS

Operational and program problems on this contract have been minimal considering the magnitude and nature of the undertaking. The need for construction, renovation and alteration has already been mentioned. Constant awareness is required to avoid expenditures for desirable, attractive adjunct facilities such as conference rooms and additional administrative space when the needs are so great to provide adequate facilities for research programs, resource production and support services. A great deal of effort has been devoted to reducing overhead or allocated costs. As the programs expand, projects are consolidated in buildings that are already active in order to avoid opening buildings now in stand-by status. These considerations require constant awareness of the disproportionate growth of space devoted to administrative functions.

Because of the nature of the contract, Award Fee, the most effective means of management is the evaluation of the contractor's performance not only in the conduct of research and production of resources, but in all phases of operation and maintenance of the Center and subsequent determination of the amount of the fee. The monitors and coordinators have essential roles in this process through their frequent communications with the managers of

individual projects and activities. They not only provide guidance but have the opportunity to observe deficiencies and to work with the contractor's managers to correct them and improve performance. Although it is difficult in some areas of research, this award fee process promotes the setting of performance standards and goals.

The hierarchy of decision-making has not been well-established, but steps are being taken in NCI to define responsibilities and limits of authority.

GENERAL RESOURCES

Administrative

At the end of the current fiscal year the staff of the FCRC will total 615 personnel. A revised Affirmative Action Plan was submitted to the regional office for review. The employment of minorities at FCRC remains at approximately 18 percent of the total staff which compares to an available work force in Frederick County of 6.3 percent.

The Technical Information Center underwent an organizational change during the year to better utilize available personnel and resources. Graphic arts and publications services have been expanded during the year while overall costs have been reduced.

The Public Information Office continued to serve the local and scientific communities with speakers, seminars, tours, news releases and written information. An audiovisual program has been developed which will replace many tours to research laboratory facilities. This program is less disruptive to the conduct of research activities.

The Scientific Library, consisting of some 15,000 books and 700 journal titles, provided support to all FCRC personnel in the acquisition and circulation of technical material. Literature and retrieval services using a full range of computerized programs (MEDLARS, MEDLINE, TOXLINE, CHEMCON, and CCALINE) are provided to research personnel. Requests have increased 200 percent from FY74.

The Computer Systems and Services Branch has developed and made operational scientific and administrative automated programs in support of Viral Oncology, Chemical Carcinogenesis, Animal Holding and Animal Breeding, payroll personnel, accounts payable, property systems, preventive maintenance, capital equipment, and cost accounting. Statistical and analytical services are also provided to researchers.

Operations

The Operations Directorate of LBI provided various support functions for all scientific, management and administrative activities. The Facility Maintenance Branch accomplished a significant amount of maintenance and repair work during the year. Renovations in Building 538 for the Viral Oncology and Chemical Carcinogenesis, in Building 539 for the Basic Research Program, and

in Building 433 for the DCT Fermentation Laboratory were the major activities supported.

The Facility Engineering and Construction Branch provided all engineering services relating to facility design, construction and renovation, and special purpose scientific equipment required at FCRC. The following major construction projects were initiated during the year:

a. Renovation of Building 433, Chemotherapy Fermentation Laboratory, Project 17

b. Renovation of Building 522, Animal Virus Laboratory, Viral Oncology

c. Construction of Animal Suite, Second Floor, North End, Building 539, Chemical Carcinogenesis

d. Renovations, East Wing, First Floor, Building 538, Endocrine Research Project, Chemical Carcinogenesis

e. Renovations, West Wing, Second Floor, Building 538, Research in Viral Oncology Safety

f. Animal Holding Facility, Building 571, Project 11

g. Construction of Fermentation Pilot Plant, Building 472, Project 17

h. Renovation of First Floor Laboratory Area, Building 539, Basic Research Program

In addition, major utility systems improvements have been engineered and are under construction in Buildings 560, 538 and 539.

The workload in the Logistics Branch has increased during the year. The Purchasing Department received and processed in excess of 9,000 requisitions for material and services during the year. A Service Supply Center was established, and issued approximately 1,000 line items per month of laboratory and office supplies.

ESTIMATED COSTS FOR OPERATION
OF FREDERICK CANCER RESEARCH CENTER, FY 1975

Participating Element	Contract	Interdepartmental Support Agreement	Maintenance & Construction	Total
Viral Oncology DCCP	$ 5,042,482	$ 1,101,844	$ 1,381,103	$ 7,525,429
Carcinogenesis DCCP	4,873,179	1,101,844	793,943	6,768,966
DCT	1,041,666	474,641	2,536,631	4,052,938
DCBD	1,251,216	474,641	549,608	2,275,465
Basic Research	1,730,564	0	943,924	2,674,488
NINDS	249,372	237,320	0*	486,692
TOTALS	$14,188,479	$ 3,390,290	$ 6,205,209	$23,783,978

*Estimated construction costs do not include $1,800,000 for facilities for Laboratory of Central Nervous Systems Studies, NINDS.

Annual Report
Smoking and Health Program
Division of Cancer Cause and Prevention
National Cancer Institute

July 1, 1974 through June 30, 1975

Introduction

The use of tobacco poses a dual demand for public health action, to promote public awareness of smoking hazards, and to reduce the risk of those who continue to smoke, by devising protective and preventive measures.

Public education campaigns have been sponsored by the National Clearinghouse for Smoking and Health, now part of the Center for Disease Control, DHEW, by the American Cancer Society, the American Heart Association and other private organizations.

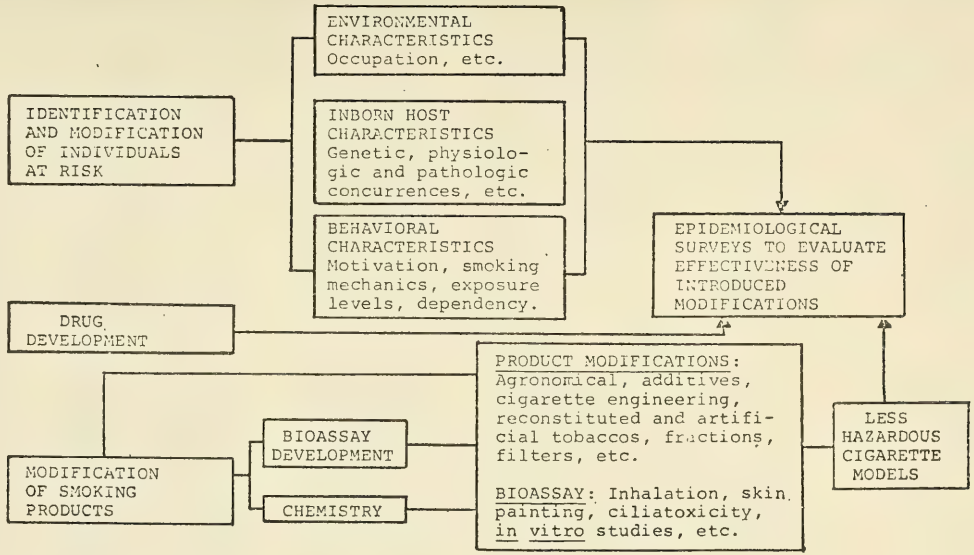
The coordinated efforts of the National Cancer Institute, the National Heart and Lung Institute and the United States Department of Agriculture, have been specifically directed at the identification of individuals most likely to develop smoking related cancers and diseases, to means of reducing their risk, and to the development of less hazardous cigarettes. A general plan of these major research approaches is shown in Chart I.

Markers of individuals at higher risk may include inborn characteristics and environmental factors such as genetic defects, physiologic and pathologic concurrences, nicotine dependency, motivation, occupation, social habits, smoke intake and smoking mechanics, etc. It is necessary to develop methods for the identification of these markers. Also, pharmacologic and clinical methodologies must be devised to help the high-risk smoker who should but finds it difficult to quit; these include the study of drugs that aid in smoke cessation or can counteract specific deleterious effects of smoking, and physiologic and motivational research in clinical approaches to smoke cessation.

Retrospective and prospective studies showing a dose-response relationship indicate that a reduction of exposure to tobacco smoke should result in reduced incidence of smoking related cancers and diseases. In fact, during the past 20 years a dramatic reduction in the amount of tar and nicotine yields of American commercial cigarettes has taken place; recent epidemiological studies suggest that a smoker today must smoke more of modern cigarettes than he would have of cigarettes available 20 years ago, in order to develop the same risk of disease, and that smoking filter cigarettes for 10 years or more significantly decreases the risk of lung cancer as compared to smokers of unfiltered cigarettes. All this points to the desirability of filter cigarettes and other low yield products on the market.

CHART I

RESEARCH PLAN TO DECREASE THE RISK OF SMOKING RELATED CANCERS AND DISEASES


ENVIRONMENTAL CHARACTERISTICS Occupation, etc.
IDENTIFICATION AND MODIFICATION OF INDIVIDUALS AT RISK
INBORN HOST CHARACTERISTICS Genetic, physiologic and pathologic concurrences, etc.
BEHAVIORAL CHARACTERISTICS Motivation, smoking mechanics, exposure levels, dependency.
EPIDEMIOLOGICAL SURVEYS TO EVALUATE EFFECTIVENESS OF INTRODUCED MODIFICATIONS
DRUG DEVELOPMENT
PRODUCT MODIFICATIONS: Agronomical, additives, cigarette engineering, reconstituted and artificial tobaccos, fractions, filters, etc.
BIOASSAY: Inhalation, skin painting, ciliatoxicity, in vitro studies, etc.
LESS HAZARDOUS CIGARETTE MODELS
BIOASSAY DEVELOPMENT
MODIFICATION OF SMOKING PRODUCTS
CHEMISTRY

Moreover cigarettes can be engineered to produce smoke which, on an equal dose basis, can produce higher or lower carcinogenic response and other pathologic manifestations in animals. Choice and manipulation of appropriate tobacco types, engineering of the cigarette and selective filtration will permit removal of a considerable portion of pathogenic smoke components, and still maintain consumer acceptability. This should result in smoking products which are intrinsically less injurious to human health, over and above the simple reduction of tar and nicotine.

The health and technological problems connected with tobacco and tobacco smoking are complex and cross a variety of disciplines and interests. For instance, it is to the advantage of tobacco farmers and a function of the USDA to foster research on agronomic varieties of tobacco that may result in less harmful smoking products, as evaluated by agencies with the necessary competence, such as the NCI and the NHLI.

The improvement of some cigarette characteristics may possibly result in worsening others; close collaboration of experts in carcinogenesis, cardiovascular and respiratory diseases and tobacco technology is mandatory to insure that cigarettes become less hazardous for as many as possible of their potential effects in man. The National Cancer Institute will also coordinate with the National Heart and Lung Institute and other public health agencies in pharmacologic research and clinical application of motivation studies leading to smoke withdrawal techniques for the high-risk smoker, and in research for the identification of individuals at risk due to inborn characteristics and environmental factors.

With the assistance of the Tobacco Working Group (the principal advisory body) and other consultants qualified in a wide spectrum of disciplines, the National Cancer Institute has developed specifications for a series of less hazardous cigarette models, as well as requirements for chemical analysis and biological assay. Carefully designed experiments are being carried out to determine the properties of these candidate less hazardous cigarette models.

A status report written in July 1974 highlighted such progress as identifying non-volatile nitrosamines and hydrazine in tobacco and tobacco smoke, the synthesis and bioassay of the isomeric methylchrysenes, demonstrating that the rat is a useful laboratory animal for smoke inhalation studies, proving feasible the grafting of excised tracheal tissue from a donor animal to the dorsal epidermis of a recipient as a means of exposing tracheal tissue to tobacco condensate, and data from epidemiological studies that show the reduced hazard from low tar - low nicotine filtered cigarettes.

Progress has continued with the initiation of efforts in inhalation bioassay in both dogs and rats, research on the aryl hydrocarbon hydroxylase as a possible marker of high risk of cancer, the development of additional capabilities in the analytical chemistry of cigarette smokes and condensates, and the adaptation of a number of pulmonary physiology tests to the dog.

In addition to the contributions of the individual projects, Dr. Gio B. Gori, the Director of the Smoking and Health Program, has recently sponsored two special workshops - one on Animal Models and End-points of Potential Use in Research on Cardiovascular Effects of Tobacco Smoke and its Components, and another on Candidate Methods for Measurement of Tobacco Smoke Components in Human Subjects.

Lists of Tobacco Working Group Members, Smoking and Health Review Board Members and consultants follow.

Tobacco Working Group

Dr. William W. Bates
Director, Research Department
Liggett & Myers Incorporated

Dr. Fred G. Bock (Chairman)
Director, Orchard Park Laboratories
Roswell Park Memorial Institute

Dr. Alfred Fishman
Director, Cardiopulmonary Division
Hospital of the University
of Pennsylvania

Dr. Jean D. Gibbons
Chairman, Department of Statistics
and Quantitative Methods
University of Alabama

Dr. Gio B. Gori
Deputy Director
Division of Cancer Cause
& Prevention
National Cancer Institute

Dr. Ian Higgins
Department of Epidemiology
School of Public Health
Observatory
University of Michigan

Dr. Dietrich Hoffmann
Chief, Division of Environmental
Carcinogenesis
American Health Foundation

Dr. Charles J. Kensler
Senior Vice President
Arthur D. Little, Inc.

Dr. Henry C. McGill, Jr.
Professor of Pathology
Health Science Center
University of Texas

Dr. Gardner C. McMillan
Chief, Arteriosclerotic
Disease Branch
National Heart & Lung Institute

Dr. Thomas B. Owen (Exec. Secy.)
Assistant to the Director of the
Smoking and Health Program
Division of Cancer Cause
& Prevention
National Cancer Institute

Dr. Murray Senkus
Director of Research
R. J. Reynolds Tobacco Company

Dr. Philippe Shubik (Liaison, NCAB)
Director, The Eppley Institute
for Research in Cancer
University of Nebraska

Dr. A. W. Spears
Director, Research and Development
Lorillard Research Center

Dr. T. C. Tso
Chief, Tobacco Laboratory
Beltsville Agricultural
Research Center
Agricultural Research Service, USDA

Dr. Helmut Wakeham
Vice President, Research and
Development
Philip Morris, U.S.A.

Smoking and Health Review Board

Dr. Joseph F. Fraumeni, Jr.
Associate Chief, Epidemiology Branch
National Cancer Institute

Dr. Harry W. Hays
Staff Scientist
Agricultural Research Center, USDA

Dr. Gardner C. McMillan
Chief, Arteriosclerotic Disease Branch
National Heart & Lung Institute

Dr. Mearl F. Stanton
Medical Officer
Laboratory of Pathology
National Cancer Institute

Dr. T. C. Tso
Chief, Tobacco Laboratory
Beltsville Agricultural Research Center
Agricultural Research Service, USDA

Smoking and Health Program Consultants

Dr. Alan Armitage
Head Pharmacologist
Council for Tobacco Research

Dr. Mark Armstrong
Department of Medicine
University of Iowa

Dr. Oscar Auerbach
Senior Medical Investigator
Veteran's Administration Hospital

Dr. James Bain
Professor of Pharmacology
Emory University

Dr. Carl G. Baker
Consultant
Hazleton Laboratories, Inc.

Dr. David Bates
Dean of Medical School
University of British Columbia
Faculty of Medicine

Dr. William W. Bates
Director, Research Department
Liggett & Myers, Inc.

Dr. Sam P. Battista
Senior Staff Pharmacologist
Arthur D. Little, Inc.

Dr. John Berg
Director, Iowa State Cancer Registry
Department of Community Medicine
College of Medicine
University of Iowa

Dr. Fred G. Bock
Director, Orchard Park Laboratories
Roswell Park Memorial Institute

Dr. Roswell K. Boutwell
Professor of Oncology
McArdle Laboratory for Cancer Research
University of Wisconsin

Dr. Edward Bresnick
Chairman, Molecular Biology
Medical College of Georgia

Dr. Kenneth Brinkhous
Chairman, Pathology Department
University of North Carolina

Smoking and Health Program Consultants (Cont'd.)

Dr. Stuart Brooks
Department of Environmental Health
Medical Sciences Building

Dr. Ercoli Cavalieri
The Eppley Institute for Research
in Cancer
University of Nebraska
College of Medicine

Dr. James F. Chaplin
Director, U.S. Department of
Agriculture
Tobacco Research Laboratory

Dr. O. T. Chortyk
Chief, Tobacco Laboratory
Richard B. Russell Research Center

Dr. Thomas B. Clarkson
Department of Comparative Medicine
Bowman-Gray School of Medicine

Dr. Morton J. Cowan
Staff Assistant to the Director
Division of Lung Diseases
National Heart & Lung Institute

Dr. Gerald Deneau
Department of Pharmacology
University of California
School of Medicine

Dr. Louis Diamond
Director, Pharmacodynamics and
Toxicology Division
College of Pharmacology
University of Kentucky

Dr. Edward F. Domino
Department of Pharmacology
University of Michigan

Dr. Grace M. Donnelly
Bioassay Project Leader
Tobacco and Health Research Institute
University of Kentucky

Dr. John Edward Dunn
Head, San Francisco Bay Area
Resource for Cancer Epidemiology
California Department of
Public Health

Dr. Ronald Estabrook
Department of Biochemistry
University of Texas
Health Science Center

Dr. Hans L. Falk
Associate Director for
Laboratory Research
National Institute of Environmental
Health Sciences

Dr. Alfred Fishman
Director, Cardiopulmonary Division
Hospital of the University
of Pennsylvania

Dr. Robert Forster
Hospital of the University
of Pennsylvania

Dr. F. John Francis
Head, Department of Food Science
& Technology
University of Massachusetts

Dr. Joseph F. Fraumeni, Jr.
Associate Chief, Epidemiology
Branch
National Cancer Institute

Dr. Jean D. Gibbons
Chairman, Department of Statistics
and Quantitative Methods
University of Alabama

Dr. Michael R. Guerin
Analytical Chemistry Division
Oak Ridge National Laboratory

Smoking and Health Program Consultants (Cont'd.)

Mr. Howard M. Halter
Director of Engineering
Chemical Division
World Tobacco Group
AMF Incorporated

Dr. E. Cuyler Hammond
Vice President for Epidemiology
and Statistical Research
American Cancer Society

Dr. Harry W. Hays
Staff Scientist
Agricultural Research Center, West

Dr. Allen Heim
Coordinator for Collaborative
Research, Carcinogenesis
National Cancer Institute

Dr. John Heller
Special Consultant for
International Affairs
National Cancer Institute

Dr. Ian Higgins
Department of Epidemiology
School of Public Health
Observatory
University of Michigan

Dr. Peter Hill
Head, Lipid Metabolism
Division of Nutrition
American Health Foundation

Dr. Marcus E. Hobbs
Department of Chemistry
Duke University

Dr. Dietrich Hoffmann
Chief, Division of Environmental
Carcinogenesis
American Health Foundation

Dr. Evan C. Horning
Professor, Institute for
Lipid Research
Baylor College of Medicine

Dr. I. W. Hughes
Director, Research & Development
Brown & Williamson Tobacco Corp.

Dr. Ronald H. Ingram
Peter Bent Brigham Hospital

Dr. Thomas N. James
Professor & Chairman
Department of Medicine
University of Alabama
Medical Center

Dr. Murray E. Jarvik
Director, Psychopharmacology Unit
VA Brentwood Hospital
(Psychosocial Medicine)

Dr. Kenneth Keller
Assistant Director for
Tobacco Research
North Carolina State University

Dr. Charles J. Kensler
Senior Vice President
Arthur D. Little, Inc.

Dr. Jerome Kleinerman
St. Luke's Hospital
Department of Pathology Research

Dr. Francis Klocke
Department of Medicine
State University of New York
Buffalo General Hospital

Dr. George B. Koelle
Professor and Chairman
Department of Pharmacology
School of Medicine
University of Pennsylvania

Dr. H. Kubota
Analytical Chemistry Division
Oak Ridge National Laboratory

Dr. Marvin Kuschner
Dean, School of Medicine
Health Sciences Center
State University of New York
at Stony Brook

Dr. Louis Lasagna
Chairman, Department of Clinical
Pharmacology
University of Rochester

Dr. Claude J. Lenfant
Associate Director for Lung Programs
National Heart & Lung Institute

Dr. William Lijinsky
Atomic Energy Commission
Oak Ridge National Laboratory

Dr. John P. Long
Department of Pharmacology
College of Medicine
University of Iowa

Dr. Owen Maller
Monell Chemical Senses Center

Dr. Henry C. McGill, Jr.
Professor of Pathology
University of Texas
Health Science Center

Dr. Gardner C. McMillan
Associate Director for Etiology
of Arteriosclerosis and
Hypertension
Division of Heart & Vascular Diseases
National Institutes of Health

Dr. Harold Menkes
Department of Medicine
Johns Hopkins University
School of Medicine

Dr. Sanford A. Miller
Professor of Nutrition
Massachusetts Institute
of Technology

Dr. E. L. Moore
Staff Scientist
National Program Staff
Beltsville Agricultural
Research Center

Dr. Kenneth Moser
University Hospital of
San Diego County
Pulmonary Division

Dr. Jay Moskowitz
Acting Chief of the Special
Programs and Resources Branch
National Heart & Lung Institute

Dr. Robert Murphy
Department of Pharmacology
University of Colorado
Medical School

Dr. Paul Nettesheim
Leader, Respiratory Carcinogenesis
Group
Oak Ridge National Laboratory

Dr. Mark Nickerson
Chairman, Department of
Pharmacology and Therapeutics
McGill University

Dr. Thomas Osdene
Director of Research
Philip Morris, U.S.A.

Dr. Osgood D. Priddle
Bausch and Lomb

Dr. James R. Prine
Los Alamos Scientific Laboratory
University of California

Dr. Donald F. Proctor
Johns Hopkins School of Hygiene

Dr. Gunter Rathkamp
Sales Manager
Waters Associates
Waters Mestenick G.m.b.H.

Dr. William K. Riker
Department of Pharmacology
University of Oregon
Medical School

Dr. F. Alan Rodgman
Section Head
Research Department
R. J. Reynolds Tobacco Company

Dr. Geno Saccomanno
Pathologist and Director of
Laboratories
St. Mary's and V.A. Hospitals

Dr. Umberto Saffiotti
Associate Director for Carcinogenesis
Division of Cancer Cause & Prevention
National Cancer Institute

Dr. Irwin Schmeltz
Head, Bio-Organic Chemistry
American Health Foundation

Dr. Marvin A. Schneiderman
Associate Director for Field
Studies and Statistics
Division of Cancer Cause & Prevention
National Cancer Institute

Dr. Frederick J. Schultz
Manager, Research
Lorillard Research Center

Dr. Irving J. Selikoff
Director, Division of Environmental
Medicine
Department of Community Medicine
Mt. Sinai School of Medicine of the
City University of New York

Dr. William Selke
Director of Research
Peter J. Schweitzer Division
Kimberly Clark Corporation

Dr. Murray Senkus
Director of Research
R. J. Reynolds Tobacco Company

Dr. A. W. Spears
Director, Research & Development
Lorillard Research Center

Dr. Mearl F. Stanton
Laboratory of Pathology
National Cancer Institute

Dr. Jesse L. Steinfeld
Veterans Administration Hospital

Dr. G. W. Stokes
Associate Dean
Department of Agriculture
University of Kentucky

Dr. Jack T. Strong
Louisiana State University
Medical School

Dr. Philip S. Thayer
Vice President
Head, Life Sciences Section
Arthur D. Little, Inc.

Dr. Wilbur Thomas
Albany Medical College

Dr. Bela Toth
Professor of Pathology
The Eppley Institute for
Research in Cancer
University of Nebraska

Dr. Richard E. Tracy
Louisiana State University

Dr. T. C. Tso
Chief, Tobacco Laboratory
Beltsville Agricultural
Research Center
Agricultural Research Service,USDA

Dr. Benjamin L. Van Duuren
Professor of Environmental Medicine
New York University Medical Center
Institute for Environmental Medicine

Dr. Helmut Wakeham
Vice President
Research & Development
Philip Morris, U.S.A.

Dr. Lee Wattenberg
Professor of Pathology
University of Minnesota

Dr. Elizabeth Weisburger
Chief of Carcinogen Metabolism
and Toxicology Branch
National Cancer Institute

Dr. John H. Weisburger
Vice President for Research
American Health Foundation

Dr. John Wyatt
Director, Tobacco & Health
Research Institute
University of Kentucky

Dr. Ernst L. Wynder
President
American Health Foundation

Accomplishments

The following paragraphs summarize the major activities and/or events which were significant to the Smoking and Health Program for fiscal year 1975.

- Methods for routine chemical analyses of tobacco smokes and condensates were extended by development of methods for neophytadiene, catechol, nitric oxide independent of other oxides of nitrogen, and adaptation of high resolution gas chromatography to visualize some 150-200 gas phase components of tobacco smoke. The neophytadiene analyses correlate well with isoprene, and thus promise to give more relevant data on the terpenoid component of condensates.

- Development of inhalation bioassays using rats has progressed with further improvements in animal restraint equipment at ORNL and Battelle. 180 rats (including controls) at ORNL have been smoking seven or more cigarettes daily for nine months with no deaths. Data on average dose in these animals are being obtained by weekly determination of nicotine depletion in the chamber during smoke exposure. Work on dosimetry is currently directed to the effects of smoke holding time vs. smoke concentration at levels of 5, 10 and 15 percent smoke using labelled dotriacontane as tracer.

- Inhalation bioassay in dogs includes efforts at the VA Hospital under Dr. Auerbach, at Hazleton Laboratories and at Battelle Northwest. The dogs at Dr. Auerbach's laboratories have been smoking for one year. Two dogs in the high nicotine group have died, one at 170 days (1004 cigarettes) and one at 269 days (2192 cigarettes). Both animals showed basal cell hyperplasia and changes in the lung parenchyma with the one receiving the larger number of cigarettes showing greater change. Hazleton

Laboratories has been able to obtain a closing volume in dogs with good reproducibility, and are also developing procedures to carry out a battery of other pulmonary physiology tests including data on single-breath nitrogen washout at maximum expiratory flow, single-breath CO diffusion capacity, residual volume, expiratory reserve volume, inspiratory capacity, maximum expiratory flow-volume, vital capacity and total lung capacity. Battelle Northwest is developing equipment and training personnel to initiate its long-term exposure of dogs to high and low nicotine cigarettes with collateral biochemical and physiologic tests.

- Analyses of the histopathological data from the First and Second Cigarette Experiments have been completed. Rankings of the cigarettes and correlation of bioassay and chemical data for these series did not change significantly from the analyses made on the basis of visual observation. The apparent discrepancy between visual observation and histopathological data was resolved; the differences can be accounted for by the sum of regressed lesions plus lesions found to be non-tumorigenic by histopathology. A report of these findings was distributed to TWG members.

- Routine smoke and condensate analyses and leaf analyses for cigarettes in the Second Cigarette Experiment have been completed at Oak Ridge National Laboratories and the USDA Agricultural Research Service (ARS), respectively. These organizations have initiated similar analyses for the cigarettes in the Third Cigarette Experiment.

- Preparation of condensates from cigarettes in the Third Cigarette Experiment by Meloy Laboratories continues as does skin painting of the condensates on mice at Hazleton Laboratories. Termination of the Third Cigarette Experiment is planned in September 1975. Life tables for each group of mice will be computed monthly during the last six months of the experiment to provide preliminary progress information.

- Cigarettes are being manufactured for the Fourth Cigarette Experiment by the R. J. Reynolds Tobacco Company. Reconstituted tobacco sheets are being prepared by AMF and Peter J. Schweitzer Division. A list of the experimental cigarettes for this series has been distributed to the TWG members. When the cigarettes are made condensate preparation and skin painting studies will be initiated.

- In a collaborative venture, the Agriculture Research Service, USDA, and the NCI have prepared approximately

1500 pounds each of Bright and Burley tobacco by a homogenized leaf curing process. This tobacco and equal amounts of normally cured Bright and Burley tobaccos have been made into sheet at AMF and cigarettes are being manufactured at Reynolds. Additionally, ARS has grown pesticide treated and untreated tobaccos on pesticide-free land on Prince Edwards Island and has processed these into cigarettes. These will provide additional agronomic and processing variables for future experiments.

- Steps have been initiated to prepare a standard set of morphometric procedures for examination of pulmonary and cardiovascular tissues in animal experimentation in the Smoking and Health Program. Adoption of these procedures should provide greater comparability of pathological data from the various contractor laboratories in the Program.

- The Monograph for the First Cigarette Experiment has been written in final form and discussions concerning publication have been initiated. A draft of the Monograph for the Second Cigarette Experiment has been sent to the Tobacco Working Group members for review.

- Proceedings of the Inhalation Workshop, held in June 1974, are in final form. Copies of this document have been sent to the Tobacco Working Group members.

- The Policies and Procedures Manual for the Smoking and Health Program has been revised, finalized, and sent to the Tobacco Working Group members.

- One subcommittee of the Tobacco Working Group met during the reporting period. The Agriculture Subcommittee chaired by Dr. T. C. Tso met in Raleigh on October 27, 1974.

- One new contract was placed at North Texas State University for standardization and improvement of the aryl hydrocarbon hydroxylase enzyme assay. Three requests for proposal were advertised for contractual efforts:

  "Pulmonary Screening Tests for the Effect of Cigarette Smoke on Small Airways in the Human Lung"

  "Clinical Trials for Pharmacological Approaches to Smoking Withdrawal"

  "Feasibility of Inhalation Testing of Cigarettes in Baboons".

CHARACTERISTICS OF LESS HAZARDOUS CIGARETTES

- Reconstituted tobacco sheets (RTS) in general resulted in condensates less carcinogenic than whole tobacco. The RTS condensate activity did not appear to be affected by the relative density of the RTS, but was affected by the removal of certain tobacco fractions or the addition of diluent additives, according to the specific manufacturing methods.

- High relative paper porosity and smoke dilution devices appear to decrease the carcinogenic activity of condensate.

- Cigarettes made solely of tobacco stems produced condensate of the lowest carcinogenic activity, as previously published. Stems differ in many ways from whole tobacco and their composition could provide guidelines for further tobacco modification.

- The relative concentration of nicotine, either in the tobacco or in the condensate, correlated with the condensate carcinogenic activity. It is unclear whether nicotine itself is carcinogenic or merely an indicator of other carcinogens, the concentration of which parallels that of nicotine.

- The relative concentration of petroleum ether solubles in tobacco correlated with the condensate carcinogenicity. Conceivably, they may have a role as precursors of condensate carcinogens.

At the present time it is likely that the trend to development of a less hazardous cigarette will thus include a larger proportion of reconstituted tobacco sheet in the cigarette blend, a reduction of nicotine content, utilization of paper of higher porosity as wrappers for the cigarettes, smoke dilution devices and some chemical manipulation or extraction of carcinogen precursors in the tobacco.

TABLE 1

SMOKING AND HEALTH PROGRAM
CURRENT CONTRACTS FY 75

ORGANIZATION	TITLE
American Health Foundation New York, New York NO1-CP3-3305	Evaluation of Carcinogenic Agents in Cigarette Smoke; Biological and Chemical Assays and Epidemiological Studies
Atomic Energy Commission Oak Ridge, Tennessee YO1-CP4-0207	Collection, Separation, and Elucidation of the Components of Cigarette Smoke
Atomic Energy Commission Oak Ridge, Tennessee YO1-CP4-0206	Tobacco Smoke Bioassay Research Project
Battelle Northwest Richland, Washington NO1-CP3-3374	Lung Pellet Implant - Cigarette Smoke Condensate
Battelle Northwest Richland, Washington NO1-CP4-3315	Inhalation Bioassay of Cigarette Smoke in Dogs: High/Low Nicotine
Battelle Northwest Richland, Washington NO1-CP4-3314	Inhalation Bioassays of Cigarette Smoke in Rats
Center for Disease Control New York, New York YO1-CP2-0205	Study of Smoking Intervention Techniques
Enviro Control, Inc. Rockville, Maryland NO1-CP3-3382	Management Support, Subcontract Fee, On-Going Subcontracts, New Project Subcontracts, Prime Contract Award, Tobacco and Cigarettes
Hazleton Laboratories Vienna, Virginia NO1-CP4-3320	Mouse Dermal Bioassay of Cigarette Smoke Condensates
Hazleton Laboratories Vienna, Virginia NO1-CP4-3310	Respiratory Impairment in Beagles Exposed to Cigarette Smoke

TABLE 1 (Cont'd.)

SMOKING AND HEALTH PROGRAM
CURRENT CONTRACTS FY 75

ORGANIZATION	TITLE
Huntingdon Research Center Brooklandville, Maryland NO1-CP4-5622	Metabolic Studies on Tobacco Smoke Constituents (HCN, NO_x, CO)
University of Maryland Baltimore, Maryland NO1-CP4-3312	Metabolic Studies on Tobacco Smoke Constituents (Nicotine)
Arthur D. Little, Inc. Cambridge, Massachusetts NO1-CP3-3284	Bioassay of the Cytotoxicity of Cigarette Smoke and Its Effects of Ciliary Function
Arthur D. Little, Inc. Cambridge, Massachusetts NO1-CP4-3316	Evaluation of Acceptability to Smokers of Less Hazardous Cigarettes
Meloy Laboratories, Inc. Falls Church, Virginia NO1-CP4-3361	Preparation and Analysis of Cigarette Smoke Condensate Samples
Microbiological Associates, Inc. Bethesda, Maryland NO1-CP4-3309	Standardization of AHH Assay as a Screening Method
New York University Medical Center New York, New York NO1-CP3-3241	Studies on Carcinogenesis Principles of Processed Tobaccos and Tobacco Smoke
North Texas State University Denton, Texas NO1-CP5-5626	Standardization of Aryl Hydroxylase Assay as a Screening Method to Determine Smoking Hazards in Man
VA Hospital East Orange, New Jersey YO1-CP4-0205	Test Effects of High and Low Nicotine Cigarettes on Male Beagle Dogs

AMERICAN HEALTH FOUNDATION (NO1-CP3-3305)

Title: Part I - Epidemiological Evaluation of Carcinogenic Agents in Cigarette Smoke

Contractor's Project Directors: Ernst L. Wynder, M.D.
Peter B. Peacock, M.D., D.P.H.

Project Officer (NCI): Dr. Gio B. Gori

Objectives: To determine carcinogenic factors, both endogenous and exogenous, in humans and to identify major etiologic factors relating to various types of cancers which may be worthy of further investigation in a laboratory setting, as well as of direct practical application. The continued purpose is to evaluate the risk associated with different smoking habits, including the type of cigarettes smoked and to identify the kinds of cigarettes that are less carcinogenic to various organs. The identification of specific carcinogenic factors should lead to the recommendation of necessary preventive measures and thus eventually lead to the reduction of such neoplasms.

Major Findings: As of September 30, 1974, 20,861 hospitalized individuals were interviewed by trained research interviewers at selected hospitals in New York City, Los Angeles, Houston, Birmingham, Miami, New Orleans, San Francisco and Chicago.

The present study continues to demonstrate a reduced risk of cancer of the lung in men, cancer of the oral cavity and cancer of the larynx among individuals smoking low-tar cigarettes for ten years or more. One of the fundamental purposes of the study, of course, is to determine what the risk will be for individuals who have smoked low-tar and low-nicotine cigarettes for 15, 20, 25 years and, finally, for those who have smoked such cigarettes for their entire lifetime.

Significant relationships were found between all types of tobacco smoking, but especially for cigarettes, for both glottic and supraglottic laryngeal cancers. Significant relationships were also found between alcohol consumption and both types of larynx cancer, but alcohol drinking appeared to increase the relative risk of laryngeal cancer only in the presence of tobacco usage. There was found a diminishing relative risk of both types of larynx cancers for ex-smokers and smokers of filter cigarettes for ten years or more, suggesting the existence of a "less harmful cigarette" for another type of cancer in man. It was shown that laryngeal cancer patients included fewer individuals with college or post-graduate education than controls.

An increased risk for pancreas cancer among cigarette smokers was also discovered, which should provide leads to biochemists to seek carcinogens for the human pancreas, either in human bile or pancreatic secretions.

Additionally, it was demonstrated that there is an increased risk among smokers of developing adenocarcinoma of the kidney. This finding, together with the determination that cancer of the renal pelvis is significantly related to cigarette smoking, should also provide valuable leads to chemists and encourage chemists to search for urinary carcinogens.

The present study has demonstrated that women smoke cigarettes lower in tar and nicotine than men and that, among men only, the use of low-tar, low-nicotine cigarettes was associated with higher levels of education. In addition, Jewish men and white men tended to smoke cigarettes lower in tar and nicotine than did non-Jewish and non-white men. These facts permit us to predict that tobacco-related cancers in the male population will become increasingly a disease of the lower educational groups.

The study continues to assess the possible hazards of smoking the "little cigars" and to substantiate preliminary findings of reduced risks of various cancers among male, long-term filter cigarette smokers.

Significance of Biomedical Research and the Program of the Institute: The NCI has the major responsibility of defining the hazards of smoking. Resources available at the American Health Foundation permit the organization to inter-relate the activities and findings of the epidemiology division with those of laboratory investigators in varied areas of specialization.

Such an inter-relationship on a day-to-day basis is necessary in order to fully develop the various epidemiological and experimental leads available through the study of environmental carcinogenesis. It is obvious that such studies need to be continued and that, in the final analysis, chemical and biological tests of "less harmful cigarettes" need to be evaluated in terms of their effect on man himself.

Proposed Course: Additional opportunities for expansion and continuation of scientific efforts by the contractor have been identified, should the funding situation improve.

Date Contract Initiated: February 24, 1970.

Current Annual Level: $740,600

ERDA-OAK RIDGE NATIONAL LABORATORY (Part I YO1-CP4-0207, Part II YO1-CP4-0206)

Title: Collection, Separation, and Elucidation of the Compounds of Cigarette Smoke. Part I -- Chemical Characterization of Cigarette Smoke. Part II -- Cigarette Smoke Bioassay Development

Contractor's Project Directors: Part I - Dr. Michael R. Guerin
Dr. James R. Stokely

Part II - Dr. Paul Nettesheim
Dr. Michael R. Guerin

Project Officer (NCI): Dr. Gio B. Gori

Objectives: Part I - To provide a maximum chemical characterization of cigarette smokes undergoing biological testing by other contractors and provide special services to those contractors as specified by the NCI.

Part II - To develop new methods for assessing the biological impact of cigarette smoke.

Major Findings: Part I - New analytical methods have been developed for the quantitative determination of phenol, o-cresol, m-cresol, p-cresol, neophytadiene, and catechol in total particulate matter and condensate. Neophytadiene was found highly correlated with isoprene ($r=0.86$) and biological activity ($r=-0.88$) for a selected series of experimental cigarettes. Catechol was found sufficiently correlated ($r=0.48$) to warrant further study. In a study of the delivery of sulfur containing constituents by filtered and non-filtered cigarettes hydrogen sulfide was found somewhat correlated ($r=-0.56$) with skin painting activity and hydrogen sulfide ($r=-0.80$) and carbon disulfide ($r=-0.84$) were correlated with ciliary clearance data.

A procedure has been devied which allows the determination of phenol, three cresols, catechol, glycerol, nicotine, neophytadiene, and five C_{16}--C_{18} free fatty acids in a single sample. The procedure, now undergoing final study, promises to massively increase analytical efficiency in the routine service function of this contract. Procedures have been developed which allow the quantitative detection of forty particulate matter constituents and two hundred gas phase constituents in a single analysis. This gas phase procedure has been found capable of detecting potentially biologically active constituents without prior knowledge of their identity.

The chemical composition of smokes delivered by the 25 experimental variants in the second cigarette experiment has been determined. The composition of smokes from the 28 variants comprising the third cigarette experiment is partially determined. Constituents determined include Total Particulate Matter, water, nicotine alkaloids, tar, hydrogen cyanide, acetaldehyde, acrolein, isoprene, formaldehyde, oxides of nitrogen, carbon monoxide, carbon dioxide, nicotine, phenol, o-cresol, m+p-cresols, palmitic acid, oleic + linoleic + linolenic acids, stearic acid, weak acids, very weak acids, indole, skatole, benz(a)anthracene, benzo(a)pyrene, and pH. A total of approximately 10,000 determinations have been carried out in the past year.

Part II - Chronic smoke inhalation studies with rats were initiated totalling 160 smoke exposed rats and 130 control animals. The first groups were started on 1/28/74. The rats are exposed intermittently for 10% smoke of either 7 or 10 cigarettes per day. Only 4 animals have died; these deaths occurred during the early part of the study. The major accomplishment of this experiment so far is that we have learned to expose rats chronically for much greater quantities of smoke

than has been possible in the past. The endpoints measured in this study will encompass pathology, clinical biochemistry, and physiological measurement, with emphasis on detection of damage to the respiratory and cardiovascular systems. Comparison of smoke deposition in rats and hamsters (of similar body weights) shows similar smoke uptake in both species. The maximum smoke dose tolerated in the course of a chronic exposure study is the same in both species.

A lower percentage of smoke tracer is found in the stomach of exposed hamsters when sacrificed by nitrogen suffocation than other techniques, further supporting our hypothesis that stomach "dose" results from violent movement and swallowing of tracer originally deposited in the head. A study now under way to evaluate the effect of exposure conditions on dose to rats has so far revealed a five-fold increase in lung dose provided by 40 second exposure to 21% smoke as compared to the standard 30 second exposure to 10% smoke. Tracer (dotriacontane-C^{14}, nicotine-C^{14}, and BaP-C^{14}) deposition in the animal has been found to agree well with measures of the decrease in concentration in the exposure chamber. Measureing depletion of constituents in the chamber thus affords a non-destructive method to determine the dose of any measurable smoke constituent received by the animals. Clinical chemical methods have been adopted or developed for determining 21 constituents or parameters in the urine and/or blood of experimental animals.

The Maddox/ORNL exposure system is in routine use in the chronic study underway here and systems have been built and supplied to other NCI contractors and associated institutions. The chronic exposure in-house is being monitored using measurements of nicotine, puff number, puff volume, and exposure time. Reproducibility has been found excellent and the data further define the exposure conditions.

Studies on heterotopic tracheal grafts are being continued. The objective is to develop the methodology to expose open tracheal grafts to fresh tobacco smoke. Various methods are currently being tested to interface the graft with the smoke generator. Studies with low doses of chemically defined carcinogens indicate that the tracheal graft is responsive to benzo(a)pyrene dose levels commonly used for initiation in the skin carcinogenesis assay.

Significance to Biomedical Research and the Program of the Institute: The National Cancer Institute has assumed much of the responsibility for defining the carcinogenic potential of cigarette smoking and steps which might be taken to reduce that potential. Chemical studies provide the methods and data by which biological activity may be related to characteristics of the smoke. Bioassay studies provide methods which will allow a more reliable definition of the relative biological activities of products under test.

Proposed Course: Part I - To continue providing the chemical data and methods necessary to characterize the products under test.

Part II - To finalize present studies for the development of a superior bioassay with additional attention to endpoints.

Date Contract Initiated: Part I - November 1969
Part II - July 1971

Current Annual Level: Part I - $420,000
Part II - $650,000

BATTELLE PACIFIC NORTHWEST LABORATORIES (NO1-CP3-3374)

Title: Research on Standardizing Lung Pellet Implantation Technique in the Rat

Contractor's Project Director: Dr. Gerald E. Dagle

Project Officers (NCI): Dr. Thomas B. Owen
Dr. Mearl F. Stanton

Objectives: To evaluate a beeswax-tricaprylin-cigarette smoke condensate lung pellet implantation technique to standardize the technique for the bioassay of select cigarette smoke condensates and to determine if such standardization may allow a shorter period for the bioassay of these condensates than other presently used bioassay techniques.

Major Findings: Osborne-Mendel rats were implanted with lung pellets through a thorocotomy incision with two different cigarette smoke condensates (CSC) with each CSC having three concentrations (1:50, 1:10, and 1:2 CSC to beeswax-tricaprylin vehicle) and each concentration having three pellet sizes (0.05, 0.1, and 0.2 ml). Sprague-Dawley rats were also implanted to compare another strain of rat. Squamous metaplasia associated with the lung pellets was more severe in the increasing size and higher concentration of condensates in rats sacrificed 4, 10, and 30 weeks after implantation. The squamous metaplasia was not appreciably different between the two CSC and did not markedly progress in the time periods examined.

Significance to Biomedical Research and the Program of the Institute: The induction of epidermoid carcinomas in the lungs of rats by implantation of relatively small amounts of cigarette smoke condensate in beeswax-tricaprylin-cigarette smoke condensate pellets in the lung (Stanton and co-workers) suggests that the lung pellet implantation technique could be used for routine cigarette smoke condensate carcinogenicity bioassay in the development of a less hazardous cigarette if the technique could be standardized. The potential advantages of this bioassay system would be that only one injection is required and the lung, the major concern in the human smoker, is the target organ.

Proposed Course: Histopathologic evaluation will proceed as the rats are periodically sacrificed after 60, 90, and 120 weeks of exposure. A recommendation will be made on the optimal size pellet and concentration. Pre-

neoplastic changes will be evaluated in an effort to shorten the bioassay.

Date Contract Initiated: June 30, 1973

Current Annual Level: $68,112

BATTELLE PACIFIC NORTHWEST LABORATORIES (NO1-CP4-3315)

Title: Inhalation Bioassay of Cigarette Smoke in Dogs: High and Low Nicotine Content

Contractor's Project Director: Dr. Gary M. Zwicker

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: (1) To develop the technique and procedures for using the beagle dog bioassay model to determine relationships of modified tobacco characteristics and cigarette composition to pathological effects. (2) To evaluate the beagle dog bioassay model comparing the effects of cigarette smoke from high and low nicotine cigarettes.

Major Findings: The project is divided into two parts. The first year, Phase I, is directed toward establishing the techniques and procedures to administer cigarette smoke to beagle dogs and application of quantitative measurements for characterization of smoke exposure and related biological effects.

The project was initiated on June 30, 1974. Facilities and techniques for exposing beagles to cigarette smoke of high and low nicotine cigarettes have been developed. The dogs have been tracheostomized and given pre-exposure clinical chemistry, hematological, and cardio-pulmonary function measurements. Cigarette smoke exposures began January 13, 1975 and will continue until Phase I smoke exposures are completed, April 7, 1975.

Significance to Biomedical Research and the Program of the Institute: One of the goals of the NCI Smoking and Health Program is to develop a less hazardous cigarette. Bioassay models are necessary to evaluate the effect of modified tobacco or cigarette composition. This project will develop the techniques and procedures for a beagle dog bioassay model. The model will be used to compare the biological effects of high and low nicotine cigarettes.

Proposed Course: Phase I smoke exposure will continue until April 7, 1975. Monthly and pre-sacrifice clinical chemistry, hematological, and cardio-pulmonary function measurements will be made as well as at termination, each dog will be given a complete necropsy; special emphasis is placed on pulmonary changes including emphysema and cardiovascular system evaluation. Following evaluation of all data, a detailed Phase II experimental protocol, using the beagle dog bioassay model to evaluate

and compare the effects of cigarette smoke from high- and low-nicotine cigarettes, will be submitted to NCI.

Date Contract Initiated: June 30, 1974

Current Annual Level: $857,900

BATTELLE PACIFIC NORTHWEST LABORATORIES (NO1-CP4-3314)

Title: Inhalation Bioassay of Cigarette Smoke in Rats

Contractor's Project Director: Dr. Alfred P. Wehner

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: We are presently in the first phase of this two-phase project. The objectives of Phase I are to acquire equipment and animals and to set up facilities; to select and test suitable restraint devices for exposing the rats to cigarette smoke and for making certain physiological function measurements before, during and after smoke exposures; to characterize smoke from each of the 5 types of cigarettes to be assayed in Phase II; to set up and validate physiological function tests for Phase II; to train, expose to cigarette smoke and test 6 groups of 10 rats under experimental conditions proposed for Phase II; to evaluate all data from Phase I and to submit a report to the National Cancer Institute (NCI) with recommendations for Phase II.

Major Findings: Phase I is in progress. Restraint devices for chronic exposure of rats to cigarette smoke and for physiological function measurements have been designed, built, and tested. The ORNL smoking machine has been redesigned to accommodate the new restraint devices. The redesigned smoking machines are now being built. Techniques to characterize cigarette smoke and methods for physiological function measurement before, during and after smoking have been developed.

Significance to Biomedical Research and the Program of the Institute: The results of this study can be compared with other bioassays to evaluate the validity of skin painting, the validity of this inhalation bioassay with rats, and other bioassay methods as a measure of the tumorigenic hazards of cigarette smoke inhalation. The study will assess the relative degree of hazard of the five cigarette types not previously tested by inhalation for other pulmonary and cardiovascular effects, as well as lung and other cancer. This study will evaluate the rat bioassay system for assessing the effects of cigarette smoke with regard to the NCI programmatic objectives to develop less hazardous cigarettes. It will develop and refine the technology and procedures for long-term exposure of rats to cigarette smoke for future cigarette smoke inhalation bioassays.

Proposed Course: Groups of 10 rats will be exposed to cigarette smoke from each of the five types of cigarettes to be assayed in Phase II. Smoke from each cigarette type will be characterized. Physiological functions will be measured before, during and after smoking to monitor smoke exposure and effects. All data from Phase I will be evaluated and a report will be submitted to the National Cancer Institute with recommendations for Phase II.

Date Contract Initiated: June 30, 1974

Current Annual Level: $489,701

AMERICAN HEALTH FOUNDATION (YO1-CP2-0205)

Title: Study of Smoking Intervention Techniques

Contractor's Project Director: Selwyn Waingrow

Project Officer: Daniel Horn, M.D.

Objectives: Investigation of the efficacy of various smoking intervention techniques and the relationships between success and failure, techniques used, and personality and smoking characteristics of subjects. Work is being conducted in three major areas: minimal interventions in routine procedures, intermediate interventions utilizing written and audio self-help procedures, and more-maximal clinic-type interventions, both group and individual. Study is intended to clarify variables indicative of success with minimal intervention as opposed to more intensive approaches.

To date, the three areas have been developed separately: minimal intervention subjects are smokers showing no particular interest in stopping smoking, while clinic subjects are relatively highly motivated. The crucial work of relating and contrasting levels of intervention is nearing completion.

Major Findings: Preliminary analyses of minimal intervention procedures have not been completed at this time. Only pilot procedures have been attempted in intermediate interventions. The maximal interventions have compared three techniques under four methods of assignment: self-selection, randomized, no-option groups, and fee groups. Throughout, group interaction techniques achieve greater end-of-treatment success rates than do individual counseling and individual counseling with hypnosis. After six months, success rates are similar for all three modalities and average twenty-two percent. Work is progressing on identifying and characterizing recidivists so that new ex-smokers at the end-of-treatment may be referred to maintenance programs to reduce and/or eliminate recidivism.

Significance to Biomedical Research and the Program of the Institute: These variables and success-failure data in a variety of interventions at a variety of intensity levels will provide much needed information along many dimensions simultaneously. Questions such as: What treatments work

best for women? What characteristics of women contribute toward their relative difficulty with certain attempts to stop? and so on, may all be fruitfully approached in such a format.

Proposed Course: Questionnaire, interview, treatment, and follow-up data are being analyzed. The variables to be studied will include: demographic (age, sex, socio-economic status, religion, education, etc.), attitudes toward smoking and toward smoking cessation, attitudes toward authority, peers, competition, orality, nurturance, etc., smoking behavior (intensity, activities associated with smoking, etc.), comprehensive medical history variables, attitudes toward preventive health care and risk-taking behaviors, and associated material related to smoking and health.

Date Contract Initiated: June 24, 1972

Current Annual Level: $80,000

ENVIRO CONTROL, INC. (NO1-CP3-3382)

Title: Research Management Services to the Smoking and Health Program

Contractor's Project Director: Mr. A. E. Hayward

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: To provide technical and managerial support. Specific tasks include: experimental design, analysis and evaluation of data from bio-assays and chemical analyses; preparation of working papers and reports for scientific conferences; preparation of technical RFPs; site visits and progress report reviews; logistic support for major interagency cooperative investigations; budget and program analysis; procedures for research advisory and evaluation boards; writing review reports on biomedical investigations.

Major Findings: As shown in the objectives, this contract is for support; not research. Accomplishments this year consist of several technical monographs on experimental less hazardous cigarettes and related subjects. Many special meetings and workshops were supported and statistical analyses performed as required. Site visits and program reviews conducted as directed by the NCI Project Officer.

Significance to Biomedical Research and the Program of the Institute: The significance of this segment is inseparable from that of the whole Smoking and Health Program, with which this contract is involved throughout: specifically, in such activities as the correlation of carcinogenicity with components of tobaccos and smoke, identification of chemical bases of carcinogenicity, elucidation of respiratory retention and dosimetry, and studies of the progression of acute bronchitis, chronic bronchitis, and emphysema. The contractor's most significant focal role is in the

recommendation of methods for bioassay, experimental design, and application and development of methods of statistical analysis.

Proposed Course: The contractor will continue present services and assume wider responsibilities during 1975. The present contract will terminate and ECI will continue its role under a Prime Contract agreement. ECI will assume Prime Contractor status for specified non-federal subcontracts, support responsibilities for Interagency Agreements; logistics for large cooperative experiments; data analyses and staff assistance to the Director, Smoking and Health Program in defining and evaluating development projects.

Date Contract Initiated: July 1, 1973

Current Annual Level: $993,112

HAZLETON LABORATORIES (NO1-CP4-3320)

Title: Skin Carcinogenesis Bioassay of Cigarette Smoke Condensates in Mice

Contractor's Project Directors: Mr. James L. Gargus
Dr. Marcelina B. Powers

Project Officer (NCI): Dr. Gio B. Gori

Objectives: To provide a standardized bioassay for the determination of the carcinogenicity of cigarette smoke condensates by repeated dermal applications in mice.

Whole smoke condensates are supplied by Meloy Laboratories under NCI Contract (NIH-NCI-69-2084). The experimental variables include tobacco of different origins; methods of cultivation, including fertilization, suckering agents, and soils, curing and aging methods, blends of tobacco plant parts such as leaves, stalks, and stems; reconstituted tobacco, cutting of tobacco, cigarette paper, and filters.

Female ICR mice are treated six times weekly with dermal applications of the whole smoke condensates dissolved in acetone. Skin tumors are recorded and measured. All animals are necropsied and tissues are fixed and preserved for histopathological examinations. Data on each animal are entered in a computer memory once monthly. Computations and printed reports from NCI provide monitoring data for continuous evaluation of the experimental results.

Major Findings: The mouse skin bioassays on the first series of 26 experimental condensates and the second series of 25 condensates have been completed. Some of these results were published (see attachment) and two additional papers are in preparation.

A third series of 31 smoke condensates was initiated in May, June, and July, 1974 and daily applications are continuing. A fourth series of dermal bioassays of approximately 30 smoke condensate variables will be initiated in March-April, 1975.

Significance of Biomedical Research and the Program of the Institute: The comparative bioassay results along with other monitoring bioassays and chemical determinations will permit evaluation of the biological effects of the variables in the different smoke condensates.

These comparative evaluations will provide data for testing and developing less hazardous cigarette formulations.

Proposed Course: Complete the third series of bioassays on 31 smoke condensates. Termination of these will be in October, November, December, 1975. Initiate the fourth series in March-April, 1975.

Date Contract Initiated: March 1, 1974

Current Annual Level: $241,095

HAZLETON LABORATORIES (NO1-CP4-3310)

Title: Respiratory Impairment in Beagles Exposed to Cigarette Smoke

Contractor's Project Director: William B. Coate, Ph.D.

Project Officer (NCI): Thomas B. Owen, Ph.D.

Objectives: The evaluation of four types of cigarettes in terms of respiratory impairment in beagles exposed to their smoke. In Phase I, the main objectives are to develop a method for the routine measurement of closing volume as an indicator of small airway disease in beagles and to recommend (with development as necessary) other routine pulmonary function tests and tests for limited nonpulmonary effects of smoke exposure with demonstration of a system and plan for routine exposure of chronically tracheostomized beagles to cigarette smoke boluses (35ml) from an automatic smoking machine.

In Phase II, the objectives are to conduct a long term exposure of groups of 40 beagles to each of four types of cigarettes and to routinely test them in vivo along with appropriate controls, for changes in pulmonary function and for changes in platelet aggregation correlated with carboxyhemoglobin levels. Serial sacrifices and detailed pulmonary and cardiovascular histopathology should permit determination of structure-function correlations. Special attention will be given to early indicators of smoking-initiated chronic bronchitis, emphysema, and hemostasis, both functionally and morphologically.

Major Findings: A nitrogen washout technique, modified from the NHLI 1973 protocol, was developed to measure closing volume in the beagle. Essentially, the technique involved controlling the inspiratory and expiratory volumes and rates of the anesthetized dog by evacuating and pressurizing respectively, a small whole-body respirator and continuously measuring the nitrogen concentration of a controlled expiration, through a cuffed endotracheal tube, from TLC to RV. Onset of Phase IV on the N_2 concentration vs volume curve on the XYY recorder was established by objective criteria and closing volume presented as a percentage of expiratory VC. A total of 18 beagles, 12 of which have been permanently tracheostomized for a pilot (Phase I) smoking study, have been tested and all have consistently manifested a clearcut Phase IV break in the N_2 washout curve.

The 12 tracheostomized beagles have begun smoking via a system incorporating an automatic cigarette puffing machine (Battista-design) which delivers 35 ml puffs through a cuffed tracheostomy tube with a pair of low pressure check valves which allows all expired smoke to exit through this tube, thereby by-passing the upper airways. The dogs display no overt reaction to smoking with deep inhalation at 30-second intervals.

Pre-smoking baseline measurements of DL_{CO}, Maximum Mid-expiratory Flow Rate (MMFR), VC, RV, ERV, FRC, and TLC were made on the 12 pilot study dogs. These parameters, as well as closing volume, will be measured monthly for five months during which time six dogs will serve as controls for six which will smoke 20 cigarettes per day (after gradual build-up). They will then be sacrificed for pulmonary histopathological examination.

Significance to Biomedical Research and the Program of the Institute: This project is part of the program for the development of a less hazardous cigarette. The selection of the beagle and the particular smoking system employed is based upon prior work in another laboratory which appeared to demonstrate profound pulmonary pathology within two years of daily smoking by beagles. Control over dosimetry and ability to conduct meaningful pulmonary function tests routinely further recommend this animal model.

Proposed Course: Phase II should be authorized as soon as practicable; a four-month lead-in is required before initiation of the large-scale (220 beagles) chronic smoking study (24 months). Meanwhile Phase I pilot study will continue and final plans for Phase II prepared.

Date Contract Initiated: June 30, 1974.

Current Annual Level: Phase I $113,063; Phase II $226,964

Huntingdon Research Center (NO1-CP4-5622)

Title: Metabolism of Tobacco Smoke Constituents

Contractor's Project Director: Dr. William F. Stephen, Jr.

Project Officer (NCI): Dr. Gio B. Gori

Objectives: 1. To prepare comprehensive, critical reviews of the scientific literature on the effects of smoking doses of nicotine, nitrogen oxides, carbon monoxide, and hydrogen cyanide on the toxicology, pharmacology, pathology, biochemistry, and metabolism in mammalian systems and the relationship of these data to smoking and human health. 2. To perform metabolic studies on the absorption, excretion, metabolism, and tissue distribution of carbon monoxide, hydrogen cyanide, and, to the extent possible, nitrogen oxides after administration of these compounds by the inhalation route at doses comparable to those administered to rats in the ORNL smoking machine.

Major Findings: The work on objective 1 has proceeded through initial computer searches and elimination of duplicate and non-relevant literature citations. It currently involves retrieval of relevant citations and searching for additional relevant citations in the bibliographies of retrieved citations.

The work on objective 2 requires development of new and refined methodologies to control the doses given and to measure the doses received and/or contained by entire rats, tissues, and body fluids after exposure. To date, methods have been developed to permit accurate dosing, quantitative simultaneous collection of blood, bile, and urine, and the measurement of total dose of gaseous compound received by the experimental rats. The mechanical portion of gas trapping systems has been worked out and specific reagents for trapping carbon monoxide and hydrogen cyanide in expired air are in development. Methods for measuring the respiratory parameters of the rat during dosing were also developed.

Significance to Biomedical Research and the Program of the Institute: Completion of objective 1 will provide a comprehensive view of the present state of knowledge of the effects of the subject compounds as related to smoking and human health. This will permit better definition of research programs to achieve the purposes of the Institute.

Objective 2 is part of a program to better define the characteristics of the rat/ORNL smoking machine model system for the experimental evaluation of the effects of smoking on health.

Proposed Course: With the exception of limited planning activities, work started on this program August 19, 1974. The literature work is proceeding apace and the metabolic studies will commence as soon as the gas trapping reagents, ORNL smoking machine, single rat doser, radiolabeled carbon monoxide and hydrogen cyanide, and rats of the proper strain become available. It is expected that developmental work will be completed and collection of biological data will commence within two months.

Date Contract Initiated: June 24, 1974

Current Annual Level: $168,615

UNIVERSITY OF MARYLAND (NO1-CP4-3312)

Title: Metabolic Studies on Tobacco Smoke Constituents -- Pharmacokinetics of Nicotine in Animals

Contractor's Project Director: Dr. Joseph Adir

Project Officer (NCI): Dr. Gio B. Gori

Objectives: To determine the rate and extent of biotransformation of nicotine and characterize its pharmacokinetics in rats after cigarette smoking.

Major Findings: A procedure has been developed for the extraction of radioactive nicotine and its major metabolites from biological fluids using column chromatography. The procedure allows for 90% or better absolute recovery. It should be noted that previously reported procedures failed to give absolute recoveries and when these procedures were attempted in our laboratory, only 50-60% absolute recovery of nicotine could be achieved. Furthermore, a technique has been perfected which allows for blood sampling in the rat using a chronically implanted cannula in the abdominal aorta. Serial blood and urine samples have been obtained after I.V. administration of methyl-^{14}C-nicotine to rats. At the time of preparation of this report, these samples are being extracted by column chromatography. Nicotine and its metabolites present in the elution solvent will be separated on TLC plates and assayed by liquid scintillation counting.

Significance to Biomedical Research and the Program of the Institute: The contract is part of the overall objective of NCI to reduce the health hazards from smoking and, especially of cigarettes. Knowledge of the overall metabolic fate and associated kinetics of the nicotine as a major component of cigarette smoke will help in achieving this objective.

Proposed Course: Upon receipt of the ORNL smoke generation/inhalation machine, studies on the pharmacokinetics of nicotine and the rate and extent of its biotransformation in rats will be undertaken as outlined in the Technical Proposal which was accepted as an integral part of this contract.

Date Contract Initiated: September 1, 1974

Current Annual Level: $48,966

ARTHUR D. LITTLE, INC. (NO1-CP3-3284)

Title: Bioassay of the Cytotoxicity of Cigarette Smoke and of its Effects on Ciliary Function

Contractor's Project Directors: Dr. Philip S. Thayer
Dr. Sam P. Battista

Project Officer (NCI): Dr. Gio B. Gori

Objectives: (1) To evaluate experimental cigarettes in 3 test systems: Effects of smoke on (a) growth and (b) phagocytic activity of in vitro cultivated cells; and on (c) ciliary kinetics and dynamics of mucus transport in the respiratory epithelium of experimental animals in vitro. (2) To develop and evaluate a model system to determine whether cigarette smoke has effects on carcinogenesis in the chicken trachea as influenced by experimental removal of the tracheal epithelium.

Major Findings: Bioassays - (1) In vitro bioassays for potency of the second set of 24 experimental NCI cigarettes have been completed for cilia, KB cells and alveolar macrophages. Results show that potency for several experimental cigarettes was significantly reduced in all 3 systems. A high degree of correlation (r = 0.6 to 0.92) was found between cilia bioassay potency and levels of certain smoke components. Similar, though somewhat less impressive, correlations (r = 0.56 to 0.74) were also observed for alveolar macrophage and cytotoxicity. (2) Studies are now in progress to characterize potencies of a third set of experimental NCI cigarettes.

Neoplasia Model - These studies were carried out to examine the long-term effects of exposing chickens to cigarette smoke alone, the carcinogen diethylnitrosamine (DEN) alone, and a combination of DEN and cigarette smoke. During the initial 20 weeks of testing, half of the animals received mechanical denudation of the trachea. Cytological examination of the exfoliated cells harvested during successive denudations showed progressive increases (followed in some cases by decreases) in nuclear atypia, active and reactive chromatin and metaplastic changes. They were most pronounced in the smoke and smoke + DEN groups, although DEN alone produced significant increases. Malignant changes were not found in cytological preparations. A small number of chickens were sacrificed after 2 years of treatment and the sections of lung, trachea and air sacs examined microscopically. In 29 controls no tumors were found, whereas malignant tumors were found in 32% of the 25 DEN-treated, 25% of the 8 smoke-treated, and 50% of the 4 that received both smoke and DEN. The tumors found were: DEN-4 adenocarcinoma, 3 bronchioalveolar carcinoma, 1 carcinoma in situ; smoked- 2 adenocarcinoma; smoked + DEN - 1 adenocarcinoma, 1 bronchioalveolar carcinoma. Tumors of the trachea have not been found among any of the different treatment groups. Impairment of ciliary clearance during the first 20 weeks by removing the epithelium from the upper half of the trachea 10 times at 2 weeks intervals has not influenced the 2 year survival. Animals surviving at the 2-year point were: Control, 29/90; DEN, 27/84; smoked, 18/42; smoked + DEN, 12/22; total, 86/238.

Significance to Biomedical Research and the Program of the Institute: The overall program for the formulation of a less hazardous cigarette needs to identify physiological responses of the respiratory tract

surface tissues to cigarette smoke of different composition.

This information is being correlated with carcinogenesis and chemical data in testing and directing modification of cigarettes towards less hazardous formulations.

Proposed Course: The three bioassay procedures are available for evaluation of subsequent sets of experimental cigarettes. The development and evaluation of the neoplastic model system are proceeding, and further experimental animals may be sacrificed during the year.

Date Contract Initiated: June 19, 1969

Current Annual Level: $104,302

ARTHUR D. LITTLE, INC. (NO1-CP4-3316)

Title: Evaluation of Acceptability to Smokers of Less Hazardous Cigarettes

Contractor's Project Director: Mr. John F. Angeline

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The objectives of the first phase of the work have been to orient the flavor profile panel to the range of flavor characteristics associated with a series of experimental products prepared under the direction of NCI. These samples were chosen by NCI to represent what, in their judgment, would be the broadest range of flavor variables. Our purpose in carrying out the work was to develop the flavor vocabulary specific for these products and to ascertain whether the protocols routinely used for the evaluation of cigarette flavor would be adequate for the samples in question.

Major Findings: The first phase of the work has been completed and our data suggest that the range of flavors which we have experienced in the first lot of cigarettes is consistent with those experienced in tobacco of differing types. The protocols which were developed over a twenty-year period appear to be readily applicable to the subject samples in question.

Significance of Biomedical Research and the Program of the Institute: Our preliminary findings suggest to us that we will be able to provide feedback data to those within NCI responsible for improving the flavor characteristics of reduced hazard cigarettes. We feel that the combination of flavor profile measurement technique, the use of a standardized flavor vocabulary and the introduction of mathematical modeling systems should permit rapid and reliable feedback of information.

Proposed Course: The next step in the work will be for representatives of the contractor project team to meet with representatives of NCI in order to tabulate the entire range of products both extant and in the near development stages for which flavor data must be generated. Following this tabulation, contractor personnel will develop a sampling model which will permit the generation of maximum data from a minimum number of flavor examinations. It is anticipated that this step can be accomplished quite quickly within the next working month. Following completion of that step, actual profile measurements will be made of selected samples and feedback methods of maximum value to NCI will be developed. Once this type of activity has been established on a routine basis, we would plan to work closely with NCI personnel in order to identify critical variables in the flavor structure of the product which could conceivably have a bearing on the ultimate acceptability or lack of acceptability of the product. All work planned at this time is of a laboratory nature involving the use of trained laboratory scientists working under control conditions.

Date Contract Initiated: June 24, 1974

Current Annual Level: $63,619

MELOY LABORATORIES, INC. (NO1-CP4-3361)

Title: Preparation and Analysis of Cigarette Smoke Condensate Samples

Contractor's Project Director: Dr. A. R. Patel

Project Officer (NCI): Dr. Gio B. Gori

Objectives: During one year period Meloy Laboratories will smoke over 8,000,000 cigarettes to produce the smoke condensate having as nearly as possible, uniform composition for use in a number of bioassay programs to be carried out under contract in the Chemical Carcinogenesis/Lung Cancer Task Force Program.

1. For each of the 25 types of cigarettes of the third series, the cigarettes are conditioned at 75 $\pm$ 2°F and 60 $\pm$ 5% relative humidity for at least 48 hours prior to being smoked on three manifold type smoking machines furnished by the National Cancer Institute. The smoke condensate collected in the glass trapping system is extracted with ACS certified reagent grade acetone. The acetone extract is concentrated under reduced pressure in a rotary evaporator at a temperature below 104°F yielding a condensate with less than 6% water. The residue is then adjusted by the addition of acetone and water (6%) to the composition, 250 or 500 mg tar per ml of condensate. The smoke condensate is then transferred into narrow mouth, amber glass two ounce bottles fitted with teflon-lined screw caps. The condensate bottles are shipped weekly to Hazleton Laboratories in a dry ice container

and also, when required, to other contractors according to the instructions of the National Cancer Institute Project Officer. At least 250 grams of dry condensate is produced from each of the 19 types of cigarettes and 450 grams of dry condensate is produced from each of the six types of cigarettes for an eight week skin-painting study.

Meloy Laboratories will also produce 420 grams of cigarette smoke condensate from five types of cigarettes every eight weeks for skin-painting studies by the American Health Foundation, New York.

During one year, Meloy Laboratories will produce at least twice 50 grams of dry smoke condensate from each of the 37 types of cigarettes for Oak Ridge National Laboratory contract directed by Dr. Guerin.

Beginning April 1, Meloy Laboratories will produce about 250 grams of cigarette smoke condensate from each type of the fourth series of cigarettes every 8 weeks for skin-painting study by the Hazleton Laboratories.

2. Meloy Laboratories carries out a quality control program of chemical analysis on all batches of cigarette smoke condensate both to insure uniformity and to characterize the product to some extent for the subsequent users.

   The following information is recorded on each batch:

   a. Mean butt length
   b. Total condensate yield and condensate yield per cigarette
   c. Moisture content
   d. Acetone content
   e. Condensate pH
   f. Nicotine content of the condensate and nicotine content per cigarette

3. During the one year period Meloy Laboratories will carry out the following analyses on each of the 62 types of the third and fourth series cigarettes:

   a. Nicotine in tobacco
   b. Nitrate in tobacco
   c. Reducing sugars in tobacco
   d. Ash
   e. Hexane solubles
   f. Potassium and sodium in tobacco
   g. Calcium and magnesium in tobacco
   h. Phosphorus in tobacco
   i. Static burning rate of cigarettes
   j. Average temperature of firezone at different butt lengths

Major Findings: This is a complex service contract, the scope of which is production of cigarette smoke condensate on schedule and involves quality control on each batch of smoke condensate.

Significance of Biomedical Research and the Program of the Institute: This contract is designed to produce the cigarette smoke condensate required for the execution of two other projects which are funded under the Lung Cancer Task Force: (1) Skin Carcinogenesis Bioassay of Cigarette Smoke Condensate, and (2) Chemical Analysis of Cigarette Smoke Condensate Samples.

Proposed Course: Activities of this contract started in April 1974 as experimental cigarettes became available. Analytical work on the unsmoked tobacco from these experimental cigarettes is being carried out. Production activities on the fourth series of NCI cigarettes will proceed on schedule.

Date Contract Initiated: June 28, 1974

Current Annual Level: $381,344

MICROBIOLOGICAL ASSOCIATES (NO1-CP4-3309)

Title: Standardization of Aryl Hydrocarbon Hydroxylase Assay as a Screening Method to Determine Smoking Hazards in Man

Contractor's Project Director: Dr. R. E. Kouri

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: To standardize the conditions allowing for the reproducible quantitation of aryl hydrocarbon hydroxylase (AHH) activity in human derived tissue. Special interest will be directed toward measuring AHH activity in cultured mitogen-activated human peripheral lymphocytes.

Major Findings: AHH activity can be readily detected in control as well as 3-methylcholanthrene-treated cultured peripheral lymphocytes. The activity is linear with respect to time of assay, linear with respect to cell concentration, and optimal at pH of 8.5. Blood samples can be collected in other laboratories (up to 3,000 miles away) and if received within a 24 hour period, the lymphocytes can be isolated, cultured and assayed for AHH activity. Determination of AHH activity from blood samples collected in other laboratories and sent to us using a blind protocol indicates: (1) the enzyme level can be reproducibly detected from multiple samples of blood taken from the same individual if assayed on the same day, but (2) samples from the same individual assayed on separate days can give quite disparate results. The major problem seems to be non-uniform mitogen-activated lymphocyte populations from which to assay. This mitogen-activation step is dependent on: (1) type of mitogen (e.g. phytohemagglutinin (PHA)-M, PHA-P and/or pokeweed mitogen),

(2) source of mitogen, (3) concentration of mitogen, (4) the lots of fetal calf serum in the culture medium, (5) the growth vessel, (6) the concentration of lymphocytes, and (7) the presence of autologous human serum in the medium. The lot of fetal calf serum is the major factor determining both constitutive and 3-methylcholanthrene-induced AHH activity in mitogen activated lymphocytes.

Significance to Biomedical Research in the Program of the Institute: The determination of the potential susceptibility of individuals to chemical carcinogenesis, in general, and to tobacco related carcinogenesis, in particular, is of obvious significance to the biomedical research program and the program of the Institute. We feel that the level of the AHH complex has the capability of determining this susceptibility. The standardization of this enzyme system as it applies to the human situation will be a major step towards fulfilling this goal.

Proposed Course: There are presently four courses of action presently being taken in our laboratory at this time. (1) The establishment of a constitutive AHH activity independent of the lot of fetal calf serum. This will depend on the use of other inducers of AHH such as phenobarbital and estradiol. (2) AHH activity should perhaps not be expressed in terms of units per viable cell, but rather in terms of units per either total DNA content, or units per rate of protein, DNA or RNA synthesis. (3) If a standardized source of fetal calf serum is required, then all the laboratories working in this area must use the same source and lot of serum. The determination of this particular lot is presently being evaluated. (4) That at least one other alternative assay be attempted. The assay suggested is the quantitation of the ability of the cultured human lymphocytes to convert benzpyrene from an organic soluble form to a water soluble form in a 24 to 48 hour period.

Date Contract Initiated: June 28, 1974

Current Annual Level: $71,947

NEW YORK UNIVERSITY MEDICAL CENTER (NO1-CP3-3241)

Title: Studies on Carcinogenic, Cocarcinogenic and Tumor-Promoting Principles of Tobacco and Tobacco Smoke

Contractor's Project Director: Dr. Benjamin L. Van Duuren

Project Officer (NCI): Dr. Gio B. Gori

Objectives: To determine the nature of the carcinogenic, cocarcinogenic and tumor-promoting agents that are known or suspected components of tobacco leaf extracts and of cigarette smoke condensate.

Major Findings: Based on our earlier studies, we have continued during the past year to examine, side by side, the tumor-promoting and cocarcinogenic activities of a number of phenols and polyphenols on mouse skin. It has become abundantly apparent from this work that not all tumor-promoting agents have cocarcinogenic activity. Whether the reverse is true, is at present not clear. This major new finding was followed up by subjecting a number of phenols, polyphenols and long-chain aliphatic hydrocarbons to mouse skin bioassays. This work showed that catechol, the most abundant phenol in cigarette smoke condensate has potent cocarcinogenic activity, with a low dose of benzo(a)pyrene as carcinogen. The same applies for pyrogallol which has not yet been definitively reported as a constituent of cigarette smoke condensate. Similar findings were obtained with certain long-chain aliphatic hydrocarbons which are known components of cigarette smoke condensate.

Significance to Biomedical Research and the Program of the Institute: Lung cancer is one of the leading causes of cancer death and the importance of cigarette smoking in the etiology of this disease is well established. Therefore, the identification of the agents responsible for the deleterious health effects of cigarette smoke is of critical importance for the ultimate reduction of cancer risk due to cigarette smoking. The identification of these agents, an understanding of their biological effects, and the mode of action can lead to their removal from tobacco and/or cigarette smoke.

Proposed Course: The examination of the tumor-promoting and cocarcinogenic activity of a variety of phenolic and related compounds is continuing. The cocarcinogenic and tumor-promoting activity of a series of alkanes, alkenes, terpenes, fatty acids and alcohols of tobacco leaf and/or cigarette smoke condensate is currently being evaluated. Efforts are continuing to develop an *in vitro* cell culture assay in order to screen for tumor-promoting and cocarcinogenic agents which are known components of cigarette smoke condensate.

Date Contract Initiated: January 1, 1975

Current Annual Level: $105,596

NORTH TEXAS STATE UNIVERSITY (NO1-CP5-5626)

Title: Standardization of Aryl Hydroxylase Assay as a Screening Method to Determine Smoking Hazards in Man

Contractor's Project Director: Dr. Elroy Cantrell

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: (a) To examine several human tissues as potential sources of biopsy material for studies of carcinogen metabolism in man, (b) to identify and select the tissue which is most advantageous with regards to safety and lack of trauma to the volunteer, suitability for metabolism studies as proposed, and ease of collection, (c) to optimize and standardize the methods

for measuring carcinogen metabolism in human tissue with reproducibility and accuracy, (d) to improve and characterize the techniques for measuring induction and inducibility of the AHH enzyme complex in man, (e) to examine alternate enzyme inducers and substrates for use in the above test systems, and, (f) suggest procedures for potential screening tests of AHH levels in man as it may relate to risk of cancer.

Major Findings: (a) Aryl hydrocarbon hydroxylase is greater in pulmonary alveolar macrophages from smokers than non-smokers. Other tissues including skin, gingiva, and buccal mucosa had very low levels of AHH activity. Cultured lymphocytes seemed to offer the greatest promise as a source for human enzyme studies. (b) We and several other labs found that the levels of constitutive and induced AHH were variable. Possible factors contributing to variation include variation between lots of fetal calf serum, variation in individual responsiveness to mitogens, and variation in toxicity of 3-methylcholanthrene to cells, and other poorly defined variables. (c) We have found that diphenyloxazole (PPO) is a potent inducer of AHH in cultured lymphocytes and can also serve as a substrate for the enzyme complex. PPO may be useful as a non-carcinogenic alternate inducer and/or substrate for the enzyme test system. (d) Cultured lymphocytes contain 16 α-hydroxylase which can be induced by estradiol and aryl hydrocarbons. An assay for inducibility and metabolism of estradiol has been developed. The capacity to metabolize estradiol may be related to risk of certain types of breast cancer.

Significance to Biomedical Research and the Program of the Institute: This contract is designed to evaluate and optimize the method for measuring carcinogen metabolism in man. Since inducibility of the enzyme complex for biotransformation of these chemicals may be related to risk of cancer, the development of a suitable method for measuring carcinogen metabolism in man would allow for development of large-scaled screening studies which can more clearly define the role of biotransformation as a risk factor for cancer. Persons at high risk could be counselled to recognize and avoid those risk factors pertinent to particular individuals.

Proposed Course: This project began in July, 1974 and some preliminary findings are described above. We shall continue the evaluation of PPO as an alternate inducer and substrate for AHH in cultured lymphocytes. The optimal conditions of the culture and assay are being developed and will require several months of work by several labs including our own.

Date Contract Initiated: July 15, 1974

Current Annual Level: $47,051

VETERANS ADMINISTRATION HOSPITAL (YO1-CP4-0205)

Title: Effects of High and Low Nicotine Cigarettes on Male Beagle Dogs

Contractor's Project Director: Dr. Oscar Auerbach

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The continuation of the program as described in the research proposal of determining the significance of the development of pulmonary and cardiovascular disease during chronic smoking using male beagle dogs.

Major Findings: The smoking machines designed by Dr. Sam Battista of Arthur D. Little, Inc., Cambridge, Massachusetts, and manufactured at Oak Ridge National Laboratories, Oak Ridge, Tennessee are being used daily on male beagle dogs to determine whether nicotine is importantly involved in the harmful effects produced by daily inhalation of cigarette smoke over an extended period of time. In January of 1974, including Control animals, 115 beagles were involved in the experiment. Fifty-five dogs are receiving high nicotine (HN) cigarettes, and fifty dogs are receiving low nicotine (LN) cigarettes with ten dogs being used as Controls.

The ADL designed machine delivers a puff volume of 35cc of smoke at twenty second intervals providing three puffs per minute per dog. The dogs started smoking at the rate of one cigarette in the morning and one in the afternoon. They appeared to tolerate this with no severe adverse effects other than excessive salivation and vomiting.

Beginning January 9, 1974 each dog, except Controls, has been smoked daily, seven days a week, including holidays. Dogs of the high nicotine (HN) group smoke only high nicotine cigarettes. Dogs of the low nicotine (LN) group smoke only low nicotine cigarettes. As of June 26, 1974 the animals now receive, according to protocol, six cigarettes in the morning and six cigarettes in the evening.

One animal (QP-42) of the high nicotine (HN) group succumbed on January 9, 1974. Necropsy examination revealed a moderate suppurative tracheitis and a bilateral suppurative bronchopneumonia. It is felt that this disease entity was due solely to the after effects of a recent tracheostomy.

A second animal (AGM-20) of the high nicotine (HN) group succumbed on June 29, 1974 after having smoked 1,004 cigarettes in 170 smoking days. A third animal (MB-30) of the high nicotine (HN) group died on October 5, 1974 following the smoking of 2,192 cigarettes in 269 days of continuous smoking.

Thus far AGM-20 and MB-30 have been extensively examined. Complete descriptions of the gross and microscopic examinations will be prepared.

Both dogs were in the high nicotine group and the dog which smoked longer, (MB-30, 269 days), showed more advanced changes. There is evidence of basal cell hyperplasia in the bronchial epithelium. There are also changes present within the lung parenchyma. Although the findings are preliminary, they definitely indicate that the dogs are receiving a good dose of cigarette smoke and that there are definitive changes related to cigarette

smoking. There are also changes within the lung tissue which indicate the damage done by the inhalation of cigarette smoke.

The smoking machine designed at Arthur D. Little Inc. which is being used continuously in the project has not been found to be trouble free. Modifications are still being made to improve its operation. Minor adjustments for calibration of puff volume and puff duration are still required on each machine following complete breakdown and cleaning of its parts, which is carried out approximately every three to five days of operation. Daily maintenance requires removal and cleaning or replacement of the syringes which act as a smoke chamber. Complete one way valve assemblies and inlet and outlet valves must have frequent cleaning of deposited cigarette tars. It has been an experience that the syringe plunger still requires daily lubrication for smooth operation. At the present time we are experimenting with what is hoped will develop into a self-lubricating syringe.

Kinking of the 3/16" teflon-smoke transfer tube has been somewhat of a problem. A recommendation by Dr. Sam Battista, of Arthur D. Little, Inc., Cambridge, Massachusetts, to place a wrapping around the teflon-smoke transfer tube spreading the stresses on the tube over a wider area proved to be of some value. A further suggestion and re-designing of the one way valve assembly, replacing the teflon tubing altogether, by Mr. Wayne L. Maddox of the Oak Ridge National Laboratories, Oak Ridge, Tennessee, provided a more favorable answer to the problem. Additional suggestions on other operational problems on the part of both of these men have continually provided the project with back-up help and expertise, which greatly contribute to the ultimate success of the project.

Another variable found creeping into the cigarette smoking studies has been the uneven delivery at times of 40 pounds per square inch of compressed air pressure which is used to drive the plunger in the pressurized syringe. This is to be corrected by the installation of pressure regulators at each air pressure outlet. Another problem has been the maintenance of a constant humidified environment in the storage of cigarettes to be used in the next day's smoking program. Minor adjustments made to the storage humidifier now provides a humidified environment of 55% ($\pm$ 5%) humidity.

The experimental animals being used in the project are constantly being monitored for indications of disease entities which could cause a variable in the outcome of the project. Early in the study several animals were found to be slightly parasitized with Ancylostoma caninum and Toxocara canis. These internal parasites were removed successfully following adequate treatment.

Additional efforts are made to keep the research specimen as comfortable as possible. This is accomplished by extending concerned care in the form of treatment of minor ailments, providing proper grooming through combing and brushing of the animals' hair coat, removing accumulated tartar from the teeth, cleaning of ear canals, keeping nails properly trimmed and offering a kind word to the animal during routine handling.

It has been noted that several of the animals while smoking have shown clinical manifestations of intoxication and hypoxia and have had to be resuscitated in order to continue smoking. This phenomenon has been more and more evident as the animals' accumulated smoking time increases.

Periodic biomedical data on both experimental and control animals will be undertaken in the very near future. Hematocrit, total and differential white blood count, platelet aggregation, blood cholesterol, blood carboxyhemoglobin and blood nicotine (or cotinine) will be carried out on a quarterly basis on collected blood samples. Consideration may also be given to other biomedical tests such as electrocardiogram and chest roentgenography. Sufficient numbers of each test will be conducted to establish normal variability so that the significance of quarterly test values can be judged as well as to establish optimum sampling times.

Significance to Biomedical Research and the Program of the Institute: It is believed that the developing pathology of the tissues of the experimental animals in the project will provide some answers as to the role that nicotine plays in possibly being a major contributor to the harmful effects of cigarette smoking.

Proposed Course: To continue the program as described in the research proposal.

Date Contract Initiated: April 1, 1972

Current Annual Level: $307,689

Projected Programs

Proposals have been received and are being evaluated for three new contracts. These are:

- Feasibility of Inhalation Testing of Cigarette Smoke in Baboons
- Clinical Trials for Pharmacological Approaches in Smoking Withdrawal
- Pulmonary Screening Tests for the Effect of Cigarette Smoke on Small Airways in the Human Lung.

If evaluations are favorable, the above contracts will be initiated in March 1975.

Additional programs being considered are:

- Prospective Epidemiological Study
- Multifactorial Epidemiological Study
- Cigarette Combustion: Computer Simulation Models

- Efforts relating to the dosimetry problem
- Efforts suggested during the Cardiovascular Workshop.

Allocation of Funds

The distribution of effort is shown graphically in the pie chart on the following page.

It is assumed that the three new contracts previously described will be initiated. Bioassays connected with development of a less hazardous cigarette account for the largest outlay of funds, followed by expenditures for research in the chemistry of cigarette smoke and condensate.

Program Management and Organization

The Smoking and Health Program is directed by Dr. Gio B. Gori, Deputy Director, DCCP, and a Program Manager, Dr. Thomas B. Owen. There is no in-house research staff; Enviro Control, Inc. (ECI) provides research management services and is developing into a prime contractor role with responsibility for many facets of the program including subcontracting.

The NCI Smoking and Health Program is subject to a three-tier review process which proceeds from broad policy and strategic considerations to specific establishment of project priorities, to detailed technical evaluations. Throughout the process, the Director of the Smoking and Health Program has the responsibility to guide the program utilizing the established advisory mechanisms in the ways most effective to meet program requirements.

- The first level of review is concerned with overall program strategy, objectives, and major lines of investigation. The Tobacco Working Group has been established to provide relevant information and guidance from the most qualified representatives of the governmental, industrial, institutional and academic sectors.

- The second level of review deals with individual projects, and its specific purpose is to establish the need and relevance of each project. This function is carried by the Smoking and Health Review Board, a group of government specialists assisted by outside consultants on an ad hoc basis where required.

- The third level of review is to provide detailed technical evaluation of individual projects (contracts). Because the Smoking and Health Program requires a diversified array of scientific

disciplines (e.g., cardiology, pathology, hematology, oncology, biochemistry, aerosol and filtration physics, physiology, engineering, analytical chemistry, plant genetics, agronomy, and numerous specialized fields of technology), it is impossible to form a standing committee of reasonable size to perform technical evaluation of all projects. Accordingly, the Director, Smoking and Health Program, utilizes the ad hoc efforts of eminent specialists to accomplish individual technical evaluations as required.

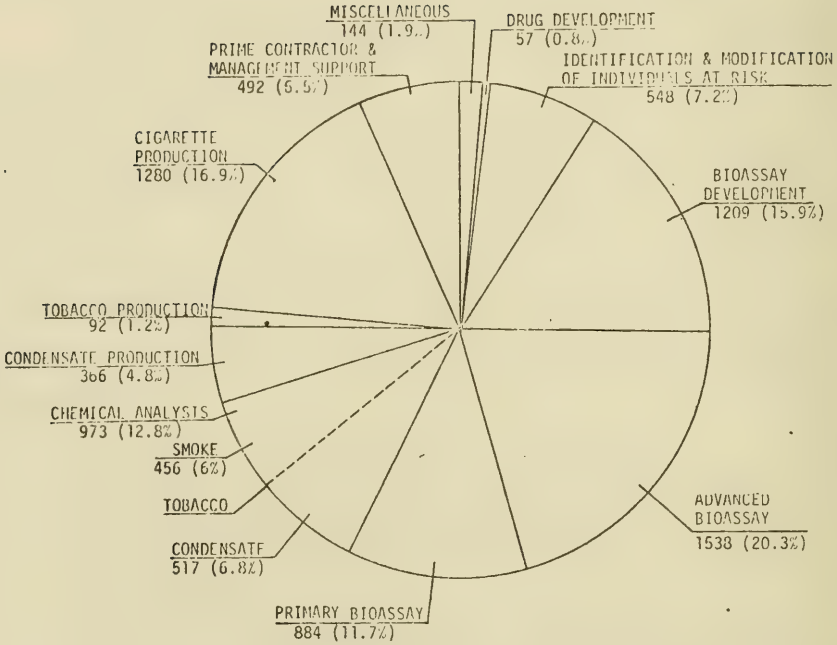
This review process is depicted in Figure 2, which also shows the relationship of various activities in the Smoking and Health Program.

FIGURE 1

CURRENT RESOURCE ALLOCATION

SMOKING & HEALTH PROGRAM

FY 1975



ALL DOLLAR FIGURES X 1,000

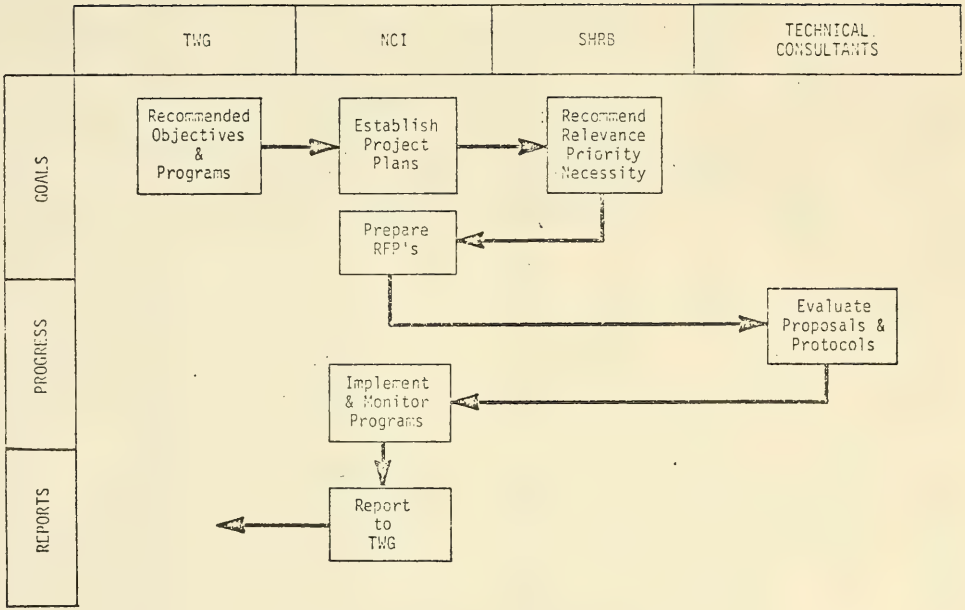
TOTAL ESTIMATED FUNDS: $7,583,000*

* Annualized Figure

FIGURE 2

RELATIONSHIP OF ACTIVITIES IN THE
SMOKING AND HEALTH PROGRAM

FLOW DIAGRAM



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SUMMARY REPORT
OFFICE OF THE ASSOCIATE DIRECTOR FOR FIELD STUDIES & STATISTICS
DIVISION OF CANCER CAUSE AND PREVENTION
July 1, 1974 - June 30, 1975

"Consider what a great girl you are. Consider what a long way you've come to-day. Consider what o'clock it is. Consider anything, only don't cry!"

Advice from the White Queen to Alice in "Through the Looking Glass" by Lewis Carroll

Our activities this year have been notable for three things:

1. The emphasis on environmental carcinogenesis.

2. The publication of two major data sets, and three summary reviews with extensive potential for use.

3. Interactions with other agencies, and other parts of the Cancer Institute at a substantially higher level than before.

ENVIRONMENTAL CARCINOGENESIS

The arguments that 70%-90% of all cancers are environmentally associated, derive from the consideration of: (1) the differential rates of cancer in different parts of the world, and (2) very rapid changes in rates among migrants, usually within one generation. The first set of considerations were first laid out by John Higginson, director of the IARC. The second set of considerations derive from the major studies on migrants conducted by William Haenszel of NCI.

The major environmental carcinogen(s) now known is contained in cigarette smoke. If one considers skin cancers (both melanoma and non-melanoma), then ultraviolet radiation is, at least for the white population, an equally important environmental "cause." The 17 confirmed human carcinogens, with two or three exceptions, are industrial materials, and account for about 5% of current cancer incidence. If one argues that almost all industrial carcinogenesis has involved men, then about 10% of cancer in men can be considered to be a result of industrial exposure. A review of 17 known chemical carcinogens, showed that possibly only aflatoxin was shown to be a carcinogen in a lower animal species before it was shown to be a carcinogen in man. This history of "finding it first in man" has important implications for future work on environmental carcinogenesis.

In the past year the FS&S Area has been actively involved in assessing the risks of aldrin-dieldrin, ultraviolet radiation, vinyl chloride, asbestos, cigarette smoke, and contaminants in public water supplies as well as conducting extensive developmental work on mathematical models for extrapolation both within an animal species, and from animals to man. The Epidemiology Branch, in particular, has reported on the relationship between the

geographic distribution of cancer mortality and various kinds of industry. The Branch has reviewed the risks of cancer in areas of Colorado where radioactive sands have been used for fill around houses, and in the Duluth area, where asbestiform particles have entered the water supply from the dumping of taconite mine tailings by the Reserve Mining company into Lake Superior at Silver Bay, about 100 miles "up lake" from Duluth. The Branch has continued to support research (in Japan, Hiroshima, Nagasaki) to elucidate the role of ionizing radiation in carcinogenesis. Members of the Area have testified at public hearings on the possible carcinogenicity of aldrin-dieldrin, vinyl chloride, and arsenic.

The other side of the "environmental" coin is the "host-factor" side. Several important pieces of work have been directed toward considering the evaluation of the immune status of a person as related to the development of cancer. Since there can be deficient immune status as a result of genetic defects as well as a consequence of drug treatments, it seems possible to develop a reasonable generalization of the effects of immune defects. At present, the cancers that arise following immune deficient status seem to be largely lymphomas, and leukemias, with some increases also in skin cancers, bladder cancers, lung cancer (adenomas) and soft tissue sarcomas. If this finding of spotty increases holds up, then there are implications about the limited role of immunity in cancer for treatment as well as for etiology that must be considered by other workers.

Other studies in host factors have been related to familial concentrations of cancer in which the Epidemiology Branch has been the leading group in the United States which have shown concatenations of several forms of cancer, and malformations and have raised issues of a general cancer diathesis among some individuals that should fit into a general theory of cancer etiology. Host-factor studies have also examined the unusual cancer incidence in certain racial groups; for example, the high rates of naso-pharyngeal, lung, liver and thyroid cancers among Chinese and the reported high rates of cancer of the esophagus in certain areas in China; unusually high rates among Alaskian natives of cancers of the nasopharynx, esophagus, kidney and salivary glands. There is some evidence of an environmental component in cancer of the nasopharynx much as there is in skin cancers, where a susceptable fair skin must be operated upon by an external environmental factor, such as sunlight.

PUBLICATIONS OF SOURCE DATA

The detailed monograph on the Third National Cancer Survey (NCI Monograph 41) is a volume almost 500 pages long with 450 different tables giving cancer incidence data in the most detailed fashion ever reported. The Monograph includes data on histologic type by site, site by histologic type as well as by the standard demographic elements of race, sex, age, and area. A public use-tape has been developed which makes all these data (on 181,027 diagnosed cancers) available at cost to qualifed research workers. A further monograph on costs will be published.

As a followup to the volume on cancer mortality by county, a set of maps, and related data are being printed in a volume entitled "Atlas of Cancer Mortality," (DHEW Publication No. (NIH) 75-780). This atlas should be available by the

time this report appears. The maps are being printed in five colors from computer generated separation negatives. With each map for each site, there is a data page giving percentile rankings of counties with both numbers of deaths and rates, a description of the map categories, a table of age-specific rates, by sex and race, and a graph of age specific rates. The computer tapes from which the maps are generated, plus the computer program for developing 5-tone white to black maps are available to qualified users. The tapes have already been made available to many users.

The "County Mortality" book has served as a basis for an RFP by the Environmental Protection Agency, calling for appropriate scientific exploitation of these data. Staff of the Epidemiology Branch assisted EPA in evaluating the proposals. In the meantime, our own staff have related the mortality data to certain known environmental and inudstrial exposures, finding high cancer rates in chemical-producing counties, counties with smelters, counties with lumbering and/or paper production, certain types of heavy machinery manufacture, and not in counties with asbestos deposits or asbestos mining. Some preliminary studies on lymphosarcoma point toward possible arsenic exposure among farmers and this lead needs to be run to earth.

In addition to these two major data sources, three other data-rich sets of materials have been published: the second edition of CANCER RATES AND RISKS, TREATMENT AND SURVIVAL PATTERNS OF BLACK AND WHITE CANCER PATIENTS (1955-1964), and RECENT TRENDS IN SURVIVAL OF CANCER PATIENTS (1960-1971). The latter volume includes one year survival data for cancers diagnosed 1970-1971; these are the most current general survival data available. The volume on Blacks and Whites shows almost uniformly poorer survival for Blacks, even when the disease is reported as "localized" at the time of first treatment. The CANCER RATES AND RISKS volume is intended for an educated, but less specialized audience, and develops a large amount of basic cancer data in a direct question and answer format.

INTERACTIONS WITH OTHERS -- AT HOME AND ABROAD

The FS&S staff have been deeply involved (sometimes entangled) with a multitude of other agencies, public, private, federal, state, international, other portions of the National Cancer Institute, and other Institutes.

With contributions from the American Cancer Society and the Division of Cancer Control and Rehabilitation, we planned, set up, managed, and ran a workshop on HIGH RISKS FOR CANCER based on a proposal by an FS&S staff member to the ACS. A volume of the papers presented at this workshop, plus a set of recommendations for future action is now in preparation for publication by the Academic Press. A further workshop on the host aspects of high risk will be held late in 1975 as a result of ideas developed at the High Risk sessions. An international workshop on the use of cancer registries in relation to environmental carcinogenesis will be held at Lyon, France in September 1975, also related to suggestions developed at the High Risk workshop.

The Food and Drug Administration is developing regulations governing the "safe" levels of materials in (animal) foods and other products based on

work conducted by the Biometry Branch. Similar work contributed to a National Academy of Sciences report on the risks of pesticides.

Staff members have testified before regulatory bodies establishing levels for aldrin-dieldrin, arsenic, vinyl chloride, etc. Research is going on in the Biometry Branch to develop better methods for extrapolating laboratory experimental data to humans. Within NCI, extensive support has been given to the Division of Cancer Control and Rehabilitation, ranging from review of RFP's to assistance in the design of screening programs, to elucidating (as much as one can) the place of the hospital-based cancer register in a cancer control program to extensive consultation on social and psychological aspects of cancer. The breast cancer screening demonstration projects have used about five man-years of FS&S professional staff. These, and other consulting activities, have strained the resources of the Biometry Branch in part because most of the NCI contractors lack adequate statistical staff and facilities. There are many contracts let with inadequate (or no) resources allotted for analysis of the data that will be generated. Consultation has been given, and developmental work has been undertaken with respect to chemical carcinogenesis, which could speed up the testing, while conducting some of it in a more real-world context.

Biometry Branch staff members have made substantial inputs into the NAS studies on the effects of changes in ozone levels on life in general, and skin cancer in particular. The time and effort spent and the questions of appropriate recognition for professional activity raise problems of a general nature that are discussed later.

In cooperation with the American Academy of Pediatrics, and the UICC, substantial international work has been undertaken. A cancer newsletter is now being circulated among pediatricians through the AAP and most recently signs bearing the insignias of the AAP and the National Cancer Program reading "For the Health of Our Children Please Don't Smoke" were distributed to 16,000 pediatricians. In cooperation with the Aemrican Cancer Society a booklet on childhood cancer statistics (including incidence and survival) was prepared and distributed. Papers on childhood cancer, in general, and Wilm's Tumor in particular have been published jointly by Biometry Branch and Epidemiology Branch staff.

The Atomic Bomb Casulty Commission has now been converted into a Japanese-run organization, the Radiation Effects Research Foundation (RERF). With the Japanese government taking on more of the support of this activity, our financial contributions have been sharply reduced. Joint activity, however, has increased. Young Japanese faculty members continue to be trained in epidemiology and to apply their training in cancer, most recently in industrial epidemiology. Recent publications from the ABCC group confirm increases in breast cancer in radiation exposed women, once again raising issues about the safety of mammography procedures used in screening.

The last major area of international collaboration has been with scientists (and others) from the Soviet Union. The second set of joint sessions with our Soviet associates took place in the United States in February 1975. At that time we agreed to two major further efforts: (1) Research in breast

cancer etiology, combining the estrogen fraction work of MacMahon and Cole with diet studies as suggested by DeWaard and Wynder, and (2) Publication of a volume (in Russian and English) on the current state of cancer epidemiology research in the two countries. Two American physician-epidemiologists have been posted to the USSR to try to find out what data are included in the Russian incidence-mortality publications, how they are collected, what are their strengths and weaknesses, and how they can be compared to U.S. data. Separate institutes in Russia have separate responsibilities with cancer incidence and mortality reporting the responsibility of the Ministry of Health, cancer treatment and survival information the responsibility of the Petrov Institute in Leningrad, and much of the laboratory and clinical research the responsibility of the Institute of Cancer Research and Clinical Oncology in Moscow. Wandering through the Red red tape is sometimes conducive to being entangled.

Migrant studies also involve international collaboration, and they have moved from the earlier descriptive epidemiology to analytic work involving collaboration with the Chemical Carcinogenesis area, and with pathologists in the United States and other countries.

PROBLEMS, PHILOSOPHY, AND PROSPECTS

The FS&S area will place substantial emphasis on the use of the CREG mechanism in the future. A set of CREG proposals was forwarded which included possible work in host-factor relations, exploitation of special populations, including veterinary populations, SEER (and other) data collections, and for the bedside education and exploitation of cancer "etiologists." Further recommendations including the followup of drug-exposed populations, and problems related to the psychosocial aspects of cancer will be forthcoming. The preliminary administrative rules concerning CREG impress us as being limiting, excessively bureaucratic and potentially inhibiting of good ideas. This would be a nice place for us to be wrong. Our attempts to exploit the VA systems as a data source have not yet been fruitful. However, the Social Security Administration has proposed using their 1% long-term employment sample and this has a good possibility for giving us some industry-wide data on mortality for comparisons among employed populations.

The issue of confidentiality (and the use of inadvertent volunteers) means a great deal to us. Rigid interpretation of the non-invasion of privacy laws will end cancer epidemiology. If we cannot identify individuals we will not be able to develop cancer incidence data (patients often go to more than one hospital or one physician. For incidence reporting we have to know who they are, so that we report them only once.) Survival data have to be recorded on individuals who must be identified if the data are to be recorded. If we are to find out if some exposure increases risk we have to know who is exposed and be able to follow him. If "informed consent" must be gotten before hospital records can be used, the withholding of consent can create biased populations, leading to wrong answers.

All these issues have been presented to the legal people in NIH and HEW who are charged with interpreting the law. So far their response has not

been helpful. At our last meeting with them, we urged them to write interpretations of the law so that epidemiologists could carry out their legitimate (and desired) business. We have not yet seen any such interpretations. The Chairman of the Biometry and Epidemiology Contract Review Committee has written to Mr. Schmidt (of the Cancer Board) expressing his committee's concern and urging appropriate action. Among other things we have pointed out is that a National Death Index would be far less invasive of privacy than the current techniques now used for followup.

The publication of the County data, and the TNCS results led us very quickly to additional problems related to freedom of information and problems of maximum useful output. The problem can be put in the form of a question "What priorities shall we assign to filling the requests of other investigators (for special tabulations, etc., for example) in contrast to the priorities we assign to our in-house analytic work?" The answer hinges, in part, on our role in the National Cancer Program and our assessment of how efforts can be most effectively used toward elucidating the cancer problem. Thus, we must ask ourselves whether it is more likely that producing special tabulations for a NAS committee (for example) will lead to more results, than following a process which will yield information for a much larger number of investigators at some later date.

So far, we have given ourselves the following answer to the question:

1. Where data are already tabulated they will be made freely available to any responsible investigator and to irresponsible ones too, with the caveat that collectors of the data will, in general, have first priority for analysis of the data.

2. Where extensive special tabulations are requested, we will make the basic data available to the people who want the special tabulations, so that they can make them, themselves.

3. We will make every effort to assist outside investigators but obviously our professional staff will not function as research assistants to outside investigators.

We are concerned about part 1 above to our answer to this question. We are concerned that persons who spend several years of their professional lives carefully collecting data shall have every opportunity to behave like professional persons and analyze the data and publish the results of their work. This is important for three reasons: (1) Without the incentive to gain and progress professionally, there will be reduced incentive to plan appropriately, and to gather the best possible data, (2) In general, the person gathering the data knows its limitations best and is less likely to make inappropriate use of the data, (3) If our professionals are to be "scooped" on the data they collect they will soon be reduced to sub-professional level.

This data issue leads into another. How shall our limited resources be deployed? The experience in environmental epidemiological research so far

has been that most carcinogens for man have been discovered by observations on man. This has dreadful public health implications for it implies that we will not discover carcinogens until a substantial population has been exposed for a long (20-30 years) time. With many " uspects" now being identified in the laboratory (and some of the labor tory tests becoming more and more remote from mammalian carcinogenesis more pressure is developing to examine every human population exposed to each new "suspect." There are serious difficulties in doing this -- the expense involved, the need for many more trained persons than now exist, and finally the inconclusiveness of the conclusions, if negative. Some ways need to be developed to achieve a balance between responsiveness to new scientific information leading to new research, and scatter-gun wasteful deployment of scarce personnel who might better and more fruitfully be doing something else. (Not least of the waste comes from inconclusive studies, which journals ofte. will not accept for publication, for good reason, which then lead the worker to ask why he is involved in doing things which are deadend scientifically and professionally and sometimes could impel him to leave the field.)

Clearly, if we are to achieve any balance between continuing existing work in an unchanging fashion, and responsiveness to new pressures, we will need to have a "quick response" capacity. This is not easy to achieve for each "quick response" in epidemiology is likely to involve several years work, tying up professional people for a long time. For example, to work on the reserpine-breast cancer issue (in cooperation with the Heart Institute and FDA) has taken two professional persons almost nine months to get the studies under way, to get OMB clearance, to write addendums to contracts, etc. and not one piece of data has yet been collected. Even with increased staff, we will have to be extremely selective in our choice of issues on which to respond, quickly, otherwise we will soon become unable to respond at all.

Some new programs, in addition to the "quick response" pressures have created additional problems for us. To pursue such an issue as the worth-whileness of BCG as a preventive for acute leukemia in children takes much time, and effort often with some resistance from the discoverers of the new technique or presumed etiology. It took substantial work to find the errors in methodology and arithmetic that now reduce the maximum BCG protective effect to 1 in 100,000 in contrast to the initial claims which were much higher. Contract programs in the Institute often do not include adequate statistical analysis of the data generated, with the burden falling on our staff. Reviews of claims related to carcinogens in Mississippi River water required weeks of work, as did the analysis of aldrin-dieldrin data, and the chlordane and heptachlor data. (A major characteristic of these experiments is their inefficient design.) There have been RFP's issued by both NCI and other institutes that have shown ignorance of work already conducted by the FS&S area requiring, then, substantial time of our professional staff in discussions with potential contractors who know of our work and sometimes for the education of project officers.

The program with the USSR will require at least one person to be full-time coordinator and editor. Such a person could probably handle relations with the Chinese, if these too should develop.

MAJOR PERSONNEL CHANGES

Mr. Mantel, our senior independent statistical consultant has retired. This is a serious loss to us. Mr. Mantel has done much work on extrapolation. We will attempt to bring on a senior-level consultant to work with us, and FDA, jointly on these problems. Dr. Cutler, the father of the SEER program, and one of the most productive statisticians in the world working on cancer problems, will also retire. Dr. John Mulvihill has returned to us from Johns Hopkins and has become head of the Clinical Genetics Section of the Epidemiology Branch. Dr. William Blot, a statistican with experience at ABCC, has joined the Epidemiology Branch. Dr. Robert Tarone, mathematical statistician, has joined the Biometry Branch. We are recruiting for a chief of the Biometry Branch, anticipating Mr. Haenszel's stepping down to conduct his research on stomach and bowel cancer. Dr. John Goldsmith of the California Health Department has been with us for two fruitful years, and is returning to California. We plan to continue to use him as a consultant in environmental epidemiology.

HONORS AND AWARDS

THOMAS R. FEARS, PH.D.

Conferences:

Annual Meeting of Aerospace Medical Association, Washington, D. C., May 6, 1974.
Conference on Evaluation of the Hepatoma in the Rodent in Carcinogenesis Bioassay, Jefferson, Arkansas, February 11-12, 1975.

Consultant:

Carcinogenesis -- Bioassay Segment on Design and Analysis of Experiments
Carcinogenesis -- Information Segment on an Epidemiological Study of N-nitroso Compounds
Skin Cancer Working Group

BERNARD H. FOX, PH.D.

Conferences:

Conference on Cancer Control & the Behavioral Sciences. Presentation: "Psychosocial Epidemiology of Cancer." San Antonio, Texas, Jan. 20-22, 1975.
National Academy of Sciences, Academy Forum, Experiments & Research with Humans: Values Coflict. Washington, D. C., February 18-19, 1975.
Working Conference on Preventing Cancer in New Mexico. Presentation: "Probable Payoff in Primary Prevention of Cancer." Albuquerque, New Mexico, March 21, 1975.

Consultant:

National Institute on Alcohol Abuse & Alcoholism
Cancer Control Program, Education & Training Branch
Division of Cancer Control & Rehabilitation: Treatment, Rehabilitation, & Continuing Care Branch; Office of Committee & Review Activities; Resources Branch
National Institute of Neurological Diseases & Stroke: Perinatal Research Br.

Committee Membership:

Education & Training Review Committee, Cancer Control Program
Ad Hoc Committee on Unsolicited Proposals, Cancer Control Program
Strategic Planning Task Force, NCI
International Committee on Alcohol, Drugs & Traffic Safety (Ass't Secy)
Committee on Alcohol & Drugs, National Safety Council
Conference Planning Committee, Conference on Cancer Control & the Behavioral Sciences, San Antonio, Jan. 20-22, 1975

HONORS AND AWARDS (Dr. Bernard Fox) [Continued]

Editor:

Co-editor with Joseph W. Cullen & Ruby N. Isom, Psychosocial Aspects of Cancer. Proceedings, Conference on Cancer Control & the Behavioral Sciences.

Publications:

Fox, B.H. and Howell, M.A.: Cancer Risk Among Psychiatric Patients: A Hypothesis. Internatl. J. Epid. 3(3): 207-208, 1974.

JOHN R. GOLDSMITH, M.D.

Conferences:

Environmental Health Board National Committee on Vital & Health Statistics, Minnesota, July 10-11, 1974.
International Symposium, "Recent Advances in the Assessment of the Health Effects of Environmental Pollution," Paris, France, June 28-July 7, 1974.
CEC-EPA International Symposium ,Paris, France, June 24-28, 1974.
Seventh International Scientific Meeting, Epidemiological Association Session VIII C, University of Sussex, Sussex, England, August 1974.
NACC Meeting, New Orleans, Louisiana, October 12-15, 1974.
International Health Conference, Reston, Virginia, October 16-18, 1974.
Symposium on Health Effects of Fossil Fuel, Indian Wells, California, November 11-13, 1974.
Air Pollution Medical Research Conference, AMA, San Francisco, California, December 5-6, 1974.
Workshop on Persons at High Risk of Cancer: An Approach to Cancer Etiology & Control. Sponsored by NCI and American Cancer Society, Key Biscayne, Florida, December 9-12, 1974.
SOEH Conference on Health Effects of Occupational Lead & Arsenic Exposure. Chicago, Illinois, February 23-25, 1975.
AMA/American Industrial Health Conference-60th Annual Meeting, San Francisco, California, April 14-17, 1975.
Air Quality Advisory Meeting, Riverside, California, March 24-26, 1975.
N.Y. Academy of Sciences Conference on Occupational Carcinogeneses, New York, March 27-29, 1975.
Preparatory Meeting for the International Conference on Cancer Registries & Occupational Cancer, San Mateo, California, May 12-13, 1975.
Czechoslovakia UNDP Project, April 20-26, 1975.
ATS/CTS Meeting, Montreal, Canada, May 18-21, 1975.
Society of Epidemiology Research Meeting, Albany, New York, June 19-21, 1975.

Consultant:

EURO-WHO On Czech Pollution UNDP Project with Reference to Air Pollution and Respiratory Disease.
Witness for Subcommittee on Health & the Environment. A statement submitted to House of Representatives Subcommittee on Health and the Environment Considering the Clean Air Act Extension.

HONORS AND AWARDS (Dr. John Goldsmith) [Continued]

Testimony on S.776, the Toxic Substances Control Act for the Senate Commerce Committee, U.S. Senate, Washington, D. C.
Presentation of testimony for NACC and ALA at EPA Hearing, Washington, D.C.
Statement of EPA at Hearing on "Health Implications of Emission from Catalytic Exhaust Device Equipped Automobiles," Washington, D. C.
Consultant Committee on Environment Health Met, San Francisco, California, October 26, 1974.

Committee Membership:

Chairman of Panel on Statistics Needed to Ascertain the Effects of the Environment on Health. (Social Security Administration)

Editorial Committee:

Editorial Review for JOURNAL OF OCCUPATIONAL MEDICINE
Editorial Review for SCIENCE, "Influence of Magnesium on the Intestinal Absorption of Lead"
Editorial Review for the NEW YORK ACADEMY OF SCIENCE, "The Identification of Risk Factors in Employed Populations"
Editorial Review for ENVIRONMENTAL RESEARCH, "Soft Tissue Lead in Baltimore Residents"

Associate Editor:

International Shipyard Health Conference. Proceedings of a Meeting Sponsored by SOEH. "Shipyard Health Problems" for publication in ENVIRONMENTAL RESEARCH.

Publications:

Goldsmith, J.R., Berglund, K.: Epidemiological Approach to Multiple Factor Interactions in Pulmonary Disease - The Potential Usefulness of Path. Analysis. Annals of the N.Y. Acad. of Sci. 221: 361-375, 1974.

Goldsmith, J.R.: Letter to the Editor of LANCET, 1974.
Goldsmith, J.R., Aronow, W.S.: Effects of Cigarette Smoking & Breathing Carbon Monoxide on Cardiovascular Hemodynamics in Anginal Patients. Circulation 50: 340-347, 1974.
Goldsmith, J.R.: Environmental Asbestoses as a Risk Factor. Western J. of Medicine 121: 419-420, 1974.

MARVIN A. SCHNEIDERMAN, PH.D.

Conferences:

NCI/ACS Executive Staff Conference, Hershey, Pa., Sept. 9-10, 1974.
"Statistical Problems in Developing Procedures for Estimating Risks to Man from the Result of Animal Experiments." Wrightsville Beach, Fla., September 29-October 2, 1974.

HONORS AND AWARDS (Dr. Marvin Schneiderman) [Continued]

Subcommittee Meeting on Geochemical Environment Related to Health and Disease. Captiva Island, Florida, October 7-8, 1974.
"Persons at High Risk of Cancer: An Approach to Cancer Etiology and Control," Key Biscayne, Florida, December 9-12, 1974.
"Safe Drinking Water Act," National Academy of Sciences, Washington, D.C., February 5, 1975.
Second Meeting of the U.S./USSR Joint Working Group on the Epidemiology of Cancer, Bethesda, Maryland, February 9-21, 1975.
Biometric Society, St. Paul, Minn., March 24-26, 1975.
Conference on Occupational Carcinogenesis, New York Academy of Sciences, New York, March 28, 1975.
ACS/NCI National Conference on Advances in Cancer Management, Denver, Colorado, May 1-3, 1975.
Pharmaceutical Manufacturers Assoc. Research & Development Section, "Benefit to Risk Evaluation in New Drug Development," Marco Island, Florida, May 5-7, 1975.
Health Effects of Chemicals in Food. Ottawa, Canada, May 12-14, 1975.
Nutrition in the Causation of Cancer. Key Biscayne, Florida, May 19-21, 1975.
Third World Conference on Smoking & Health, New York, June 2-5, 1975.

Committee Membership:

Adjunct Professor, Biostatistics, University of Pittsburgh
Clinical Associate Professor, Medical Statistics, Georgetown University School of Medicine
Member, Diagnostic Research Advisory Group, NCI
Member, Council for Analysis & Projection, ACS
Chairman/Executive Secretary of the Committee of Presidents of Statistical Societies (COPSS)
Member, Board of Directors, ASA
Member, Executive Committee of the Biopharmaceutical Subsection, Biometric Society
Councillor-at-Large of the Society for Occupational & Environmental Health
Member, Advisory Board, Research Support Center, V.A. Hospital
Coordinating Committee on Protection of Privacy, NIH
Consultation to the Editorial Board of the JOURNAL OF CHRONIC DISEASE
Member, Science Advisory Board, NCTR
Member, Epidemiology Advisory Committee, University of Southern California
Consultative Panel on Hazards of Chemical Pesticides, NAS/NRC
Associate Member, Graduate Faculty, Georgetown University, Washington,D.C.
Member, Task Force on Aging Populations, NCI
Member, Cancer Center Advisory Committee, Howard University, Washington,D.C.

Lectures:

Juniata College, Huntingdon, Pa., March 17, 1975.
Medical College of Virginia, Richmond, Virginia, April 2, 1975.
Millersville State College, Millersville, Pa., April 16, 1975.

HONOR AND AWARDS (Dr. Marvin Schneiderman) [Continued]

Associate Editor:

CANCER RESEARCH
BLOOD

Publications:

Schneiderman, M.A.: Safe Dose? The Problems of the Statistician in the World of Trans-Science. J. Wash. Acad. of Sci. 64(2): 69-78, 1974.
Schneiderman, M.A.: Letter to the Editor. On Historical Controls. Medical World News 15(34): 12, Sept. 1974.
Schneiderman, M.A.: Letter to the Editor. Phenobarbitone and Liver Tumours. The Lancet, No. 7888, p. 1085, Nov. 2, 1974.
Schneiderman, M.A.: Digestive System Cancer Among Persons Subjected to Occupational Inhalation of Asbestos Particles: A Literature Review with Emphasis on Dose Response. Envir. Health Perspectives 9: 307-311, 1974.
Schneiderman, M.A.: How Do You Know You've Done Any Better? Cancer 35(1): 64-68, 1975.
Schneiderman, M.A., Mantel, N. and Brown, C.C.: From Mouse to Man -- or How to Get From the Laboratory to Park Avenue and 59th Street Annals of the N.Y. Acad. of Sci. 246, p. 237, 1975.

Serial No. ZO1-CP-04405-01-0ADFSS
1. Office of the Associate Director for FS&S, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Bender-Gestalt Visual-Motor Test Studies in Cancer and Other Patients

Previous Serial Number: None

Principal Investigator: William Regelson, M.D. (Med. College of Virginia)

Other Investigators: Bernard H. Fox, Ph.D.; J. Robert Butler, Ph.D.

Cooperating Units: Medical College of Virginia; North Texas University

Man Years:

Total	: .5
Professional	: .4
Other	: .1

Project Description

Objectives:

To test ability of the Bender-Gestalt to distinguish cancer and other patients, or cancer stages, when given before biopsy.

Methods Employed:

The test was earlier given routinely as part of a study on cancer palliation. Data on those tested in the study will be analyzed for discrimination between cancer and non-cancer patients, matching sex and race, and correcting for age by covariance methods.

Major Findings:

Preliminary data showed evidence of perceptual-motor deficits among patients with established cancers, but these were not corrected for age, nor were they sex or race controlled. Incomplete data in the main analysis indicate age as an important source of found differences. Firm conclusions are not yet possible from the cases already tested.

Significance to Biomedical Research and the Program of the Institute:

Ability to discriminate with this test might contribute to other data on stage, and could, in some cases, identify those with occult cancer. Failure to dis-

criminate would indicate that brain damage associated with cancer is either not widespread enough for this test to be useful or does not appear until late stages.

Proposed Course of Project:

Final subjects are being run and all testing should be complete by June, 1975. Full data analysis should have been completed by fall.

Date Initiated: May 1974

Current Annual Level: $3,400 (one year)

Serial No. ZO1-CP-04406-01-OADFSS
1. Office of the Associate Director for FS&S, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Discrimination of Cancer and Non-cancer Patients by Neuromuscular Disturbances Evidenced in Handwriting

Previous Serial Number: None

Principal Investigator: Samuel Litwin, Ph.D. (Institute for Cancer Research)

Other Investigators: Bernard H. Fox, Ph.D. and Daniel G. Miller, M.D.

Cooperating Units: Institute for Cancer Research, Philadelphia, Pennsylvania, and Strang Clinic, New York

Man Years:

Total	.06
Professional	.03
Other	.03

Project Description

Objectives:

To test, under rigorous experimental conditions, the capability reported in a number of papers of distinguishing those with cancer from those without by neuromuscular responses evident in handwriting.

Methods Employed:

A group of cancer patients and other patients from Strang Clinic will submit handwriting samples. These will be analyzed blind by a person trained to identify the presumed discriminating signs and checked independently, also blind, by another such person. The results will be analyzed by site and a number of other variables.

Major Findings:

Incomplete

Significance to Biomedical Research and the Program of the Institute:

If positive findings merge in this, as well as a confirming larger study, an attempt would be made to discriminate cancer patients with occult, non-symptomatic lesions from others, in a prospective study. Also, stage relation

to the neuromuscular signs would be investigated. If negative findings emerge, this would be deemed almost definitive evidence that could lay to rest the use of that particular claim.

Proposed Course of Project:

An experimental design is being laid out, and subjects are now being selected. The handwriting samples should be completed by late fall of 1975 and the analysis by winter.

Date Initiated: October 1974

Serial No. ZO1-CP-04407-01-OADFSS
1. Office of the Associate Director for FS&S, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Evaluation of Single and Double Cervical Smears

Previous Serial Number: None

Principal Investigator: Bernard H. Fox, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	.11
Professional	.10
Other	.1

Project Description

Objectives:

To analyze the relative values of single and double cervical smears and to estimate the payoff of each in the actual screening situation.

Methods Employed:

A model of rate of discovery of true positives and true and false negatives for both cases was developed. Costs for each case will be determined and payoff per unit of cost estimated.

Major Findings:

Preliminary calculations show an important improvement in payoff for double smears over single.

Significance to Biomedical Research and the Program of the Institute:

It is expected that increased costs for double smears will not increase in proportion to the increased payoff. However, even if they increased as much as the increase in payoff, indications are that the lag in finding dysplasias and cancers in-situ will be reduced by double smears, overall preference still resting with the double smears. If benefit-cost ratios turn out as anticipated, a substantial improvement in cervical screening procedures would result.

Proposed Course of Project:

Preliminary equations have been worked out and some calculations made. These will be completed. Costs and specification of screening procedures will be explored and final calculations made. The project should be completed by fall, 1975.

Date Initiated: October 1974

Serial No. ZO1-CP-04402-OADFSS
1. Office of the Associate Director for FS&S, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Effect of Information Available to the Pathologist Who Reads Slides of Animal Tumors in Carcinogenesis Testing

Previous Serial Number: NCI-4402

Principal Investigator: Thomas R. Fears, Ph.D. and Bernard H. Fox, Ph.D.

Other Investigators: None

Cooperating Units: Carcinogenesis, DCCP

Man Years:

Total	.08
Professional	.08
Other	.01

Project Description

Objectives:

To determine the effect of the pathologist's partial and full awareness of background and treatment information in the experiment.

Methods Employed:

Appropriate experimental design, without unusual features.

Major Findings:

Significance to Biomedical Research and the Program of the Institute:

We hope to establish which of the above level(s) of information for slide reading achieve(s) maximum accuracy and minimum variation of slide reading in animal carcinogenesis experiments, thus improving their efficiency.

Proposed Course of Project:

Planning is still in progress. A previous experimental design has been changed and a new design is being constructed. An amendment to an existing contract is planned for one phase of this work and an independent contract

for the other phases. It should be ready for contract letting in late fall, 1975 and be completed by the end of FY 1976.

Date Initiated: September 1973

Serial No. Z01-CP-04408-01-OADFSS
1. Office of the Associate Director for FS&S, DCCP
2. Bethesda, Maryland

PHS-NIH
Indidividual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mis-attribution of Smoking-Withdrawal Symptoms in Sensory Deprivation

Previous Serial Number: None

Principal Investigator: Bernard H. Fox, Ph.D.

Other Investigators: Thomas I. Myers, Ph.D., American Institutes for Research, Washington, D.C.

Cooperating Units: American Institutes for Research, Washington, D.C.

Man Years:

Total	:	.08
Professional	:	.07
Other	:	.01

Project Description

Objectives:

To investigate the possibility of mis-attribution of symptoms by smokers during sensory deprivation to the deprivation rather than to not smoking.

Methods Employed:

Data previously collected during prolonged sensory deprivation without smoking will be analyzed. Pre-experiment manifest anxiety, reports of symptoms and reports of missing smoking will be related. After statistically controlling for anxiety and distress-proneness, evidence of many withdrawal symptoms together with low degree of missing smoking would suggest mis-attribution of the symptoms to the sensory deprivation.

Major Findings:

Incomplete

Significance to Biomedical Research and the Program of the Institute:

If it is found that mis-attribution did occur, this may explain the apparent success of sensory deprivation in reducing and eliminating smoking in an unusually high proportion of subjects. It would also lead to deliberate

attempts to increase success in eliminating smoking by contriving mis-attribution in smoking cessation programs.

Proposed Course of Project:

Data on 171 subjects with seven-day sensory deprivation have been collated and data on 64 others with four-day sensory deprivation are being collated. Analysis of the data will be carried out during the spring and summer of 1975. An attempt will be made to locate control subject data, for other analyses of interest. These analyses will be added to the research plan if control data are found.

Date Initiated: June 1974

Current Annual Level: $2,400 (one year only)

Serial No. Z01-CP-04404-02-OADFSS
1. Office of the Associate Director for FS&S, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Psychiatric Illness and Cancer Risk

Previous Serial Number: NCI-4404

Principal Investigator: Bernard H. Fox, Ph.D. and James E. Norman, Jr., Ph.D.

Other Investigators: None

Cooperating Units: National Academy of Sciences

Man Years:

Total	: .12
Professional	: .10
Other	: .02

Project Description

Objectives:

To establish the relation of length of hospitalization for mental disease in VA hospitals to mortality from cancer.

Methods Employed:

Literature review and evaluation, and analysis of World War I veterans' records.

Major Findings:

Evidence has been collected from the literature indicating that length of mental hospital stay may bear a strong relation to cancer mortality.

Significance to Biomedical Research and the Program of the Institute:

If it should be established that in this population, length of hospital stay is related to cancer mortality, an epidemiologic correlate in a special group may be suspected. The usually attributed reason for the found relation of length of hospital stay and cancer mortality is entrance into hospital because of possible cancer-related symptoms, both mental and physical, and since these are often associated with aged mentally ill, a similar relationship for people whose mental illness occurs long before cancer symptoms could occur indicates another variable operating.

Proposed Course of Project:

A fairly thorough review of the literature has been made and a hypothesis published (see above findings). We are preparing a proposal to NAS to carry out the work proposed in the objectives, but a limited review of the records is being conducted to find out whether the data are available and collectible. Further avenues of investigation will be pursued in the private sector if this one is not feasible.

Date Initiated: August 1973

CONTRACT NARRATIVE
OFFICE OF THE ASSOCIATE DIRECTOR
FOR FIELD STUDIES AND STATISTICS
DIVISION OF CANCER CAUSE AND PREVENTION

Fiscal Year 1975

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-43342)

Title: Etiology of Esophageal Cancer in the Caspian Littoral of Iran

Contractor's Project Director: Dr. Calum S. Muir

Project Officer (NCI): Dr. Marvin A. Schneiderman

Objectives: To carry out case-control studies of esophageal cancer in the Caspian Littoral (provinces of Mazandaran, Gilan, and Ardebil district). These studies are to include a detailed nutritional survey, examination of water supplies, crops grown, occupation, etc. Patients (and/or surviving relatives) will be interviewed and compared with matched controls.

Major Findings: 1. No simple explanation of the increased risk has been uncovered in the major suspects: bread, sheep's milk, or the "pregnancy" diet. No volatile nitrosamine has been found in sufficient excess to account for the large differences in incidence. 2. Adequate statistical surveys of an isolated, largely illiterate or semi-literate population can be conducted effectively. 3. The need for very close interaction between the laboratory and the epidemiologist was made most clear, e.g. lab techniques are now needed for measuring non-volatile nitrosamines.

Significance to Biomedical Research and the Program of the Institute: Data collection and detailed statistical analyses of epidemiological studies are almost always long-term. It is anticipated that these studies will provide information on the etiology of cancer of the esophagus (which is increasing rapidly in the black population of the United States) that can be used to prevent this cancer.

Proposed Course: Work is underway to identify possible carcinogens present in foods and drinking water. Relations with other forms of cancer will be developed.

Additional data collection and detailed statistical analyses remain to be accomplished in the areas of (a) population-sampling for personal, social and nutritional practices, (b) surveys of household and village customs and practices, as well as agricultural and other economic activities; and (c) further analytical chemistry on food samples already collected.

Date Contract Initiated: January 1, 1973

Current Annual Level: $185,388

CONTRACT NARRATIVE
OFFICE OF THE ASSOCIATE DIRECTOR
FOR FIELD STUDIES AND STATISTICS
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1975

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO (NOI-CP-33327)

Title: Group Testing for Screening Carcinogens

Contractor's Project Director: Dr. Robert M. Elashoff

Project Officer (NCI): Dr. Thomas R. Fears

Objectives: To develop mathematical techniques based on the theory of group testing to permit more rapid and efficient testing of chemicals for potential carcinogenesis.

Major Findings: 1. Formulated and evaluated a two stage, one at a time, design and compared it to a two stage group screening design and to a one stage, one at a time design. The two stage, one at a time yields important savings in sample size over the current animal bioassay test design. 2. Group screening designs have been reformulated with simpler models and decision rules. 3. Delivery group effects among negative controls have been demonstrated up to the fifty-second week of the test confirming the previous analysis of subchronic experiments.

Three reports were prepared 1) Group screening designs when screening for tumorigens, 2) a two stage sequential procedure for a decision about the binomial parameter p with an application in chemical screening, and 3) the analysis of subacute weights.

Significance to Biomedical Research and the Program of the Institute: Carcinogenesis testing is a long-term expensive process. With over 20,000 materials to test for carcinogenicity unless more efficient methods are developed testing will take a very long time. This research holds promise for more rapid testing, and for yielding information on interactions of materials.

Proposed Course: Continued close collaboration with groups doing biological testing of the materials to speed the development of mathematical models.

Development of one at a time two stage testing scheme.

Development of further applications of group testing.

Analysis and monitoring of long-term experiments.

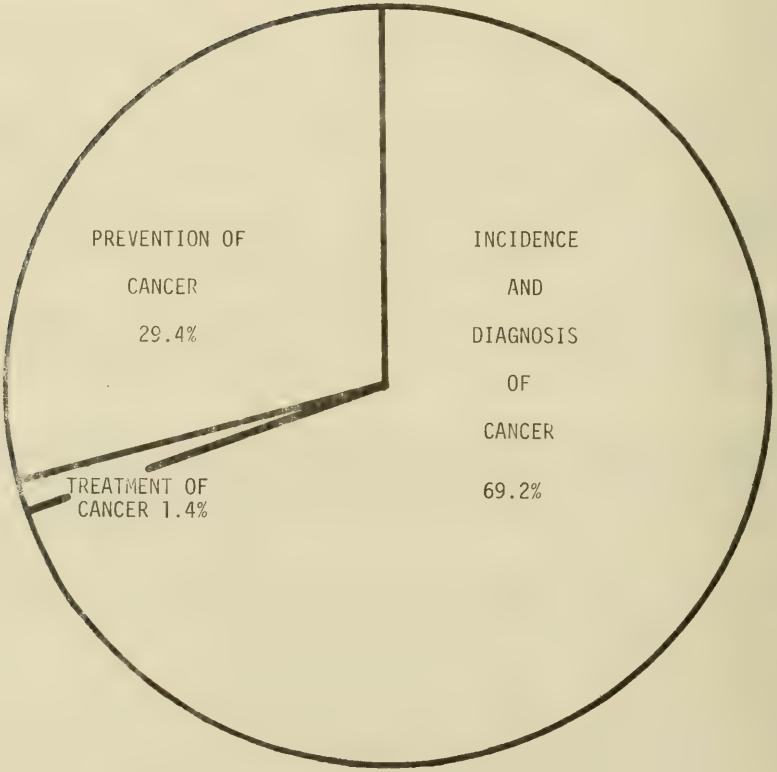
Date Contract Initiated: June 24, 1971

Current Annual Level: $99,669

DISTRIBUTION OF CONTRACT FUNDS

FIELD STUDIES AND STATISTICS

FY 1975



TOTAL CONTRACT FUNDS = $ 6,627,118

TABLE I

ANALYSIS OF CONTRACT ACTIVITIES
FIELD STUDIES AND STATISTICS

FY 1975

TYPE OF ACTIVITY	NO. OF CONTRACTS	ANNUAL LEVEL[1/]	PERCENT OF FUNDS
TOTAL:	34	$ 6,627,118	100
I. Prevention of Cancer	11	$ 1,948,015	29.4
II. Incidence and Diagnosis of Cancer	22	$ 4,586,045	69.2
III. Treatment of Cancer	1	$ 93,058	1.4

1/ Funds obligated during FY 75. Not included in this amount are ongoing activities for which no FY 75 funds were obligated.

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY	CONTRACT	DOLLAR LEVEL
I.	Prevention of Cancer	
	Aichi Cancer Center (Japan) (NO1CP23213)	$ 120,873
	American Cancer Society (NO1CP43213) - no cost extension	
	California, Univeristy of (NO1CP33327)	74,752
	Fundacion Para La Educacion Superior (Colombia) (NO1CP33286)	80,000
	International Agency for Research on Cancer (NO1CP43342)	146,000
	Kuakini Hospital and Home (NO1CP33216)	497,876
	Louisiana State University (NO1CP53521)	114,780
	Minnesota, University of (NO1CP33325)	20,715
	Norwegian Public Health Service (NO1CP43247)	100,000
	Padua, University of (Italy) (NO1CP33359)	25,000
	Research Corporation of the University of Hawaii (NO1CP53511)	454,193
	Yale University (NO1CP33235)	313,826
		$1,948,015
II.	Incidence and Diagnosis of Cancer	
	California Department of Health (NO1CP33353)	1,048,029
	California, University of (NO1CP43383)	112,973
	Case Western Reserve University (NO1CP43201)	7,590
	Charity Hospital of Louisiana (NO1CP43262)	198,021
	Commonwealth of Puerto Rico (NO1CP43386)	50,000
	Connecticut Department of Health (NO1CP43203)	277,459
	Fred Hutchinson Cancer Research Center (NO1CP33246)	425,000
	Harvard University (NO1CP33326)	90,000
	Health Insurance Plan of Greater New York (NO1CP43278)	122,172
	Iowa, University of (NO1CP43200)	379,507
	Israel Center for Registration of Cancer and Allied Diseases (NO1CP33351)	30,000
	JRB Associates, Inc. (NO1CO55426)	25,000
	Litton Bionetics, Inc. (NO1CB23227)	32,243
	Medical Association of Georgia (new)	92,398
	Meloy Laboratories (NO1CP43216)	141,878
	Michigan Cancer Foundation (NO1CP43264)	468,842
	Minnesota, University of (NO1CP43384)	25,000
	National Academy of Sciences (NO1CP43336)	160,000
	National Academy of Sciences (NO1CP43202)	184,763
	New Mexico, University of (NO1CP33344)	347,632
	Utah, University of (NO1CP43382)	290,448
	Wolf Research and Development Corporation (NO1CP33254)	77,090
		$4,586,045

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY	CONTRACT	DOLLAR LEVEL
III. Treatment of Cancer		
	Mason Research Institute (NO1CP53524)	$ 93,058
	GRAND TOTAL	6,627,118

TABLE III

ALPHABETICAL LIST OF CONTRACTS AND ACTIVITY DESCRIPTION

CONTRACTOR	FUNCTIONS	DOLLAR LEVEL
OFFICE OF ASSOCIATE DIRECTOR		
California, University of (NO1CP33327)	Prevention	$ 74,752
International Agency for Research on Cancer (NO1CP43342)	Prevention	146,000
BIOMETRY BRANCH		
Aichi Cancer Center (Japan) (NO1CP23213)	Prevention	120,873
American Cancer Society (NO1CP43213)	Prevention	no cost extension
California Department of Health (NO1CP33353)	Incidence & Diagnosis	1,048,029
California, University of (San Francisco) (NO1CP43383)	Incidence & Diagnosis	112,973
Charity Hospital of Louisiana (NO1CP43262)	Incidence & Diagnosis	198,021
Commonwealth of Puerto Rico (NO1CP43386)	Incidence & Diagnosis	50,000
Connecticut Department of Health (NO1CP43203)	Incidence & Diagnosis	277,459
Fred Hutchinson Cancer Research Center (NO1CP33246)	Incidence & Diagnosis	425,000
Fundacion Para La Educacion Superior (Colombia) (NO1CP33286)	Prevention	80,000
Health Insurance Plan of Greater New York (NO1CP43278)	Incidence & Diagnosis	122,172
Iowa, University of (NO1CP43200)	Incidence & Diagnosis	379,507
Israel Center for Registration of Cancer and Allied Diseases (NO1CP33351)	Incidence & Diagnosis	30,000

TABLE III

ALPHABETICAL LIST OF CONTRACTS AND ACTIVITY DESCRIPTION

CONTRACTOR	FUNCTIONS	DOLLAR LEVEL
Kuakini Hospital and Home (NO1CP33216)	Prevention	$ 497,876
Louisiana State University (NO1CP53521)	Prevention	114,780
Mason Research Institute (NO1CP53524)	Treatment	93,058
Medical Association of Georgia (New)	Incidence & Diagnosis	92,398
Michigan Cancer Foundation (NO1CP43264)	Incidence & Diagnosis	468,842
Minnesota, University of (NO1CP33325)	Prevention	20,715
New Mexico, University of (NO1CP33344)	Incidence & Diagnosis	347,632
Norwegian Public Health Service (NO1CP43247)	Prevention	100,000
Research Corporation of the University of Hawaii (NO1CP53511)	Prevention	454,193
Utah, University of (NO1CP43382)	Incidence & Diagnosis	290,448
Wolf Research and Development Corporation (NO1CP33254)	Incidence & Diagnosis	77,090
Yale University (NO1CP33235)	Prevention	313,826
EPIDEMIOLOGY BRANCH		
Case Western Reserve University (NO1CP43201)	Incidence & Diagnosis	7,590
Harvard University (NO1CP33326)	Incidence & Diagnosis	90,000
JRB Associates, Inc. (NO1CO55426)	Incidence & Diagnosis	25,000
Litton Bionetics, Inc. (NO1CB23227)	Incidence & Diagnosis	32,243

TABLE III

ALPHABETICAL LIST OF CONTRACTS AND ACTIVITY DESCRIPTION

CONTRACTOR	FUNCTIONS	DOLLAR LEVEL
Meloy Laboratories (NO1CP43216)	Incidence & Diagnosis	$ 141,878
Minnesota, University of (NO1CP43384)	Incidence & Diagnosis	25,000
National Academy of Sciences (NO1CP43336)	Incidence & Diagnosis	160,000
National Academy of Sciences (NO1CP43202)	Incidence & Diagnosis	184,763
Padua, University of (Italy) (NO1CP33359)	Prevention	25,000

SUMMARY REPORT
BIOMETRY BRANCH
July 1, 1974 - June 30, 1975

While the manner in which the Biometry Branch carries out its work changes in response to the needs of the National Cancer Institute and the National Cancer Program, its 3 basic functions remain unchanged: a) conduct of research on the etiology of cancer in man; b) provision of statistical consultation and support to other research investigators; c) dissemination of baseline data against which administrators can measure their successes and failures in reducing cancer incidence and mortality. These functions encompass a wide range of scientific and managerial skills in bringing people and data together.

Third National Cancer Survey

Field work for the Third National Cancer Survey, conducted in 7 metropolitan areas, 2 states and Puerto Rico and which registered all new cancer cases diagnosed in the years 1969-1971, has come to an end and all field offices were closed as of August 31, 1974, except for Detroit, Iowa and the San Francisco Bay Area which are now participants in the Surveillance, Epidemiology and End Results Program. All data have been processed and stored on tapes and staff of the Demography Section have been carrying out data analyses. A Committee on Utilization with representatives from the National Cancer Institute, contractors participating in the Survey, chronic disease epidemiologists and medical care analyst has met to offer guidance in developing a well rounded program of publications. A Public Use computer tape containing basic data on the 181,027 incidence-resident cases of cancer is available for purchase by qualified researchers and institutions.

The basic incidence data for the 3-years 1969-71 have been published in NCI Monograph 41. This publication presents data on the 181,027 cancers classified according to both primary site of the cancer and histologic cell type. This cross-classification is particularly useful to investigators who are studying specific site-histology complexes. The monograph also contains detailed incidence data for each geographic area covered in the Survey. A second monograph reporting on the incidence of non-melanoma skin cancer is being planned.

The Third National Cancer Survey data have been extensively utilized in the second eidition of Cancer Rates and Risks. Survey data have also beed used by the American Cancer Society in preparation of their annual Cancer Facts and Figures. Analyses of Survey incidence data by socioeconomic status, marital status, and urban-rural residence is under way. Analysis of cancer incidence among children, geographic variation of cancer incidence and trends in cancer incidence over time have already been published and/or presented at scientific meetings. Also, analyses of sample data with respect to patients hospitalization costs, occupational history, morbidity history, and social habits are currently being conducted.

SEER Program

Implementation of the National Cancer Program requires information that can be supplied only by a network of cancer registries. The national goal of research and action directed toward prevention, diagnosis and treatment requires information on the nature and magnitude of the total cancer problem and its components to evaluate progress. The SEER Program which has initiated new population-based registries in the United States with NCI support is a response to this need. The representation of registries in the SEER Program is designed to give wide goegraphic coverage and to include substantial representation of minority populations (Blacks, Spanish-Americans, American Indians, Japanese, Chinese). The Connecticut and California registries as SEER participants will continue to provide data on cancer incidence and patient survival. Two areas in the Third National Cancer Survey--Detroit, state of Iowa--have been transformed into a continuing registry system and will become important components of the SEER Program. The cancer registries in Utah and New Mexico are converting operations so that they will generate incidence data and serve as centers for epidemiologic research. Registries in Seattle, Hawaii and New Orleans are still in the developmental phase but should become operational as SEER members in 1975.

The Biometry Branch has been able to undertake the national coordination of the SEER network of registries because of its past experience in collaboration with the End Results Group. However, coordination of work directed to cancer epidemiology and more sophisticated studies of prognostic factors envisioned for the population-based cancer study centers and the Cancer Research Centers require new mechanisms to facilitate exchange of information to enable the Centers to develop programs compatible with broad national goals. It has been suggested that the SEER Program be reviewed by an outside committee, the review to encompass not only the objectives of the Program, but the procedures now used to meet these objectives. It is hoped that the review will develop a set of priorities for modifications that have the most favorable benefit-cost ratios.

End Results

The major emphasis of the End Results component of the Surveillance Epidemiology and End Results Program (SEER) is to analyze and publish descriptive information on cancer therapy and patient survival. After the SEER Program has been in operation long enough to accumulate several years of survival experience, it will be possible to document survival for some sub-groups of the U.S. population, e.g., Blacks, American Indians, Japanese, etc. In the meantime, some SEER participants who had previously been a part of the End Results Program are continuing to provide survival data on patients diagnosed in past years so that survival trend information is still being generated although on a somewhat reduced scale.

Branch staff has been striving to develop more meaningful ways to describe extent of disease and diagnostic procedures for cancers of the breast, colon and rectum. In collaboration with non-NCI experts, an extensive coding system and associated abstracting and coding instructions for this important aspect of patient survival analysis was implemented by all SEER participants in

January 1975. Similar descriptive schemes and instructions are being prepared for lung and bronchus, prostate, uterine cervix, uterine corpus and stomach.

Research being carried out within the section encompasses multivariate analysis of prognostic factors for several forms of cancer such as colo-rectal cancer and Hodgkin's Disease. Analytical problems posed by these efforts have stimulated development of statistical procedures for multifactor evaluation of survival. The interaction of theory development and analysis has produced advances in both areas: more meaningful analysis of data resulting from newly developed statistical methodology and data analysis providing the stimulus for developing analytical procedures based on more realistic assumptions. Another example of this same type of interaction is the development of statistical theory for evaluation of screening programs for early detection of disease. Analysis of data arising from a well designed breast cancer screening study has illustrated the need for expanding the associated statistical theory although existing methods are providing useful estimates of lead time gained by screening and estimates of the probability of detecting preclinical disease.

Active collaboration with other divisions of the NCI as well as with national and international groups has continued. With the Epidemiology Branch, examination of survival data for Wilms' tumor in children indicated that the observed increase over time in survival was consistent with the time patterns of constant incidence and decreasing mortality. In collaboration with the American Cancer Society, a professional education booklet describing childhood cancer incidence, survival and mortality was prepared. Data compiled from the National Center for Health Statistics (mortality), The Third National Cancer Survey (incidence) and the End Results Program (survival), provided estimates of the number of new cases and deaths per year currently expected among U.S. children and variation in survival by tumor type. Plans for an investigation of variation in survival for cancers of the large bowel and breast among countries exhibiting considerable differences in incidence involving collaboration with tumor registries in Norway, Finland, and Birmingham, England are being developed. The latter will take full advantage of the detailed Extent of Disease abstracting and coding procedures developed by Branch staff.

Clinical and Diagnostic Trials

The Clinical and Diagnostic Trials Section continues to provide the Veterans Administration Cooperative Urological Research Group with a complete spectrum of supporting statistical services. In return the Section has access to the largest set of data available on the course of prostatic carcinoma, and this resource also provides the Section with a substantial background of valuable experience in protocol design, data recording, collection and editing and analyses of data from clinical trials. The studies comprise an on-going series of investigations begun in 1960. Two new trials will be undertaken in 1975 and patients in all previous trials are being followed for 15 years or until death. This relationship provides a suitable environment for defining numerous general methodological studies appropriate to the analysis of data from clinical and diagnostic trials. The Section also provides consultant services to clinicians and scientists in the National Cancer Institute and other institutions.

Consultation

The Mathematical Statistics and Applied Mathematics and the ADP Management Sections continue to provide a broad range of consultation in experimental design and systems analysis to scientists in all divisions of NCI and their collaborators supported by research contracts. While these functions have been effectively discharged during the past year, some changes in the nature of problems receiving consultation deserve mention. The conventional type of statistical consultation concerned with the analysis of data from a single designed experiment is now encountered less frequently. There is now much greater demand for consultation on statistical and data handling aspects of large research-contract supported programs for which NCI staff serve as project officers. The need for this type of consultation is straining the Branch resources, and the problem has been aggravated by the retirement of the Branch's most senior and experienced consultant, Mr. Mantel, during the past year. It is quite evident that the demand for these expert services has increased in the absence of adequate statistical consultation facilities in the contractors' staffs. The relatively small NCI group cannot meet present and future needs generated by NCI research contracts and more emphasis in the award of research contracts should be placed on the ability of the contractors to hire statisticians and allied specialists to service contract-supported work. Despite these outside pressures of statistical consultation the Sections have continued to be productive in the development of new and improved techniques of experimental design and analytical techniques suitable for the investigation of problems of carcinogenesis.

Several staff members remain active as members of site-oriented task forces. Mr. Haenszel serves as a member of the Epidemiology Committee of the Breast Cancer Task Force and as a member of the Working Cadre of the National Large Bowel Cancer Project. Dr. Cutler is a member of the Steering Committee of the Breast Cancer Task Force. Dr. Byar is a cadre member of the National Prostate Cancer Project and a member of the Diagnosis and Treatment Committee of the Breast Cancer Task Force.

Migrant Studies

The Branch's study of migrant populations oriented to changes in site-specific cancer risks remains focused on migrants from Japan to Hawaii and on intra-country migration to Colombia. Although stomach has not been the target of site-oriented cancer research in the United States, substantial strides have been made in the epidemiology of stomach cancer during the past 15 years, most of it attributable to the program of migrant studies initiated by the Biometry Branch. New life has been infused into this subject, the great virtue of the migrant population approach being its ability to correlate observations over a wider spectrum of environmental exposures in home and host populations, which have clarified the following features of stomach cancer:

a. the critical role of exposures in early life in determining the level of risk

b. the important distinction between intestinal and diffuse types of the disease for descriptive and analytical epidemiology

c. documentation of intestinal metaplasia as a candidate precursor lesion

d. detection of associations with certain classes of foods that had escaped attention in studies confined to sedentes

In turn, a foundation has been laid for the transformation of stomach cancer epidemiology into the epidemiology of intestinal metaplasia and allied precursor conditions. Animal work to test associations revealed by epidemiological studies is needed, particularly the development of suitable animal models for intestinalization of the gastric mucosa. Continued pursuit of these lines of attack may yield information on how the chain of events leading to stomach cancer can be interrupted.

The epidemiological findings on large bowel cancer derived from migrant studies have laid a foundation for portions of the program of the Colon Cancer Segment of the Carcinogenesis Area and the Large Bowel National Cancer Project. They have served to narrow the area of search for etiologic factors and assist in the planning of research on the complex sequence of events leading to bowel cancer. Recent studies of intestinal polyps in high- and low-risk populations for large bowel cancer continue to reinforce impressions on concomitant population variations in intestinal polyps and large bowel cancer. Stemmermann and Yatani have described the increased prevalence and change in anatomical distribution of adenomatous and hyperplastic polyps that has accompanied a marked rise in colon cancer risks among the Japanese migrating to Hawaii. One point which has emerged with increasing clarity is the need for close coordination in the work of epidemiologists and animal experimenters.

Future Plans

The Biometry Branch in developing and monitoring contract-supported research has endeavored in the past to identify project officers knowledgeable and competent in the subject matter areas under investigation. The continued expansion of the contract program without an increase in intramural staff will force adoption of another approach. The best solution appears to be vigorous implementation of the Cancer Research Emphasis Grant Program. The latter recognized that the solution lies in decentralization of much of the ongoing analytical epidemiology activities to the population-based study centers. The latter, in turn, depends on expanding the pool of epidemiologists and biostatisticians interested in cancer epidemiology to staff the study centers. The national pool of manpower engaged in these activities is small and totally incommensurate with program needs. A vigorous program of training is urgently needed and the opportunities for training presented within the framework of the SEER Program and population-based cancer study centers should be used for this purpose.

The Third National Cancer Survey provides data useful for epidemiological studies and the planning and evaluation of cancer control programs. The End Results studies are identifying promising leads on prognostic factors for

breast and other cancer sites. Exploitation of these materials call for close collaboration with staff in the Division of Cancer Control. The pitfall to be avoided here is for the Branch not to be submerged by providing direct statistical service support to the Division of Cancer Control The Division has need for such services, but they are best met by establishing their own service unit, leaving the Biometry Branch to furnish consultation and to act as a bridge for communication between the SEER and Control Programs.

One noteworthy aspect of the migrant studies has been a shift from descriptive to analytic epidemiology to develop and test specific hypotheses. The latter has encouraged closer working relationships between Biometry Branch staff and experimentalists in the Carcinogenesis Area. This trend should continue and lead to even closer coordination in planning and execution of epidemiologic-experimental approaches to cancers of the digestive system.

Serial No. Z01-CP-04250-10-B

1. Biometry Branch, OADFS&S
   Division of Cancer Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Policy Planning and Liaison with Other Organizations

Previous Serial Number: Same

Principal Investigators: William Haenszel and Sidney Cutler

Other Investigators: James Murray, David Levin, Charles Brown

Man Years:

Total:	2.0
Professional:	1.5
Other:	0.5

Project Description

Objectives:

The principal objectives are to provide general planning and direction for the scientific program of the Biometry Branch, coordination and collaboration with other major program components of the National Cancer Institute, and active liaison with other national and international organizations.

Methods Employed:

Standard methods of program planning, management, demography, biometry and epidemiology are applied to coordinate the Branch activities and conduct the research projects.

Major Findings:

Development of the National Cancer Program has reinforced the need for an informational network within the United States, with links to international sources of information on cancer. A network of population-based cancer reporting systems providing information on a total population of more than 20 million people has been organized known as Program of Cancer Surveillance, Epidemiology, and End Results Reporting (SEER). The SEER Program coordinated by the Biometry Branch provides a base for a comprehensive cancer data program for the United States, including input from cancer centers, and data both for planning and evaluating cancer control activities. In the development of the SEER Program, particular attention has been given to the collection of descriptive information of clinical interest, e.g., referral patterns,

diagnostic procedures in assessing the neoplasm, extent of disease, treatment, and end results. The availability of information on all residents of defined geographic areas will provide data for comparison of different regions and population groups, and will provide a basis for comparing the experience of cancer centers and other specialty institutions with the experience of "the general run of patients". A working committee has been organized to promote compatibility of information from various sources. The guidelines being developed for information exchange within the United States should be very helpful in developing guidelines for exchange of compatible data on cancer on an international level.

Collaboration with various national and international bodies concerned with cancer reporting systems and cancer data has been an important element in Dr. Cutler's activities. He is a member of the Executive Committee of the American Joint Committee on Cancer Staging and End Results Reporting and a member of a WHO working group on international guidelines for End Results Reporting and coordinated the contributions of USA cancer registries to the 3rd edition of Cancer in Five Continents to be published by the International Agency for Research on Cancer. Dr. Cutler has participated in several workshops of the Association of American Cancer Institutes on exchange of information and end results reporting.

A particular effort is being made to develop information that will be useful in planning and evaluating cancer control activities. The SEER Program has been accepted as a conceptual model for cancer reporting systems that are to function as integral components of Community-based Cancer Control Programs.

The directors of the Comprehensive Cancer Centers have agreed that the basic data to be collected under the auspices of cancer centers are to be compatible with the data collected in the SEER Program. The technical materials developed for the SEER Program (abstracting instructions, codes, computer systems) are being made available to the cancer centers for their use and adaptation.

The end of the data collection phase of the Third National Cancer Survey, coupled with initiation of the SEER Program which consolidates the collection of incidence and survival data, has led to some realignments in Branch organization. A Field Liaison Section headed by Mr. Geller was created to centralize the channels of communication with the groups participating in the SEER Program. The Demography and End Results Sections headed by Drs. Young and Myers respectively are now concerned primarily with data analysis. The SEER Program has substantially upgraded the responsibilities of project officers for the several contracts. The responsibilities are viewed as primarily scientific, rather than managerial, in nature and a deliberate policy of giving all professional staff, including junior members, project officer assignments has been followed. These duties will grow in succeeding years with development of the National Cancer Program.

The collection and processing of data in the Third National Cancer Survey, covering the 3-year period, 1969-71, is complete. Attention is now being given to detailed analysis and dissemination of the available information. An advanced report for the total survey and for each of the nine areas has been

prepared and distributed by each of the local field offices. A detailed monograph containing information on both primary site and histologic type classified by race, sex, age and geographic area is being printed during March, 1975 at the Government Printing Office for national distribution this Spring.

A series of papers on individual sites, groups of sites and selected subjects have been published in individual journals and others are being prepared. Detailed analysis by socioeconomic status, marital status and urban-rural residence is currently being conducted. Plans are being made to publish a monograph containing detailed data on non-melanoma skin cancers. Data collected on a 10-percent sample of patients are being analyzed. These data include information on duration of hospitalization, repeat hospitalizations, treatment received, cost of hospital care, and sources of payment, occupational history, prior illnesses, and personal habits such as tobacco and alcohol consumption. Thus, the publication of the voluminous basic data will be supplemented by a series of interpretive analyses on individual subjects.

Several staff members continue to work as members of site-oriented task forces. Mr. Haenszel serves as a member of the Epidemiology Committee of the Breast Cancer Task Force and as a member of the Working Cadre of the National Large Bowel Cancer Project. Dr. Cutler is a member of the Steering Committee of the Breast Cancer Task Force. Dr. Byar is a cadre member of the National Prostate Cancer Project and a member of the Diagnosis and Treatment Committee of the Breast Cancer Task Force.

Dr. Levin has worked on analysis of the Third National Cancer Survey patient interview data and on the SEER Program and on problems of design of clinical trials, epidemiology of pancreatic and esophageal cancer, environmental factors in the etiology of cancer, and studies of migrant populations. He has coordinated a revision of the booklet "Cancer Rates and Risks". Dr. Levin is a member of the Medical Oncology Contract Review Committee and the Interagency Collaborative Group on Environmental Carcinogenesis; he has worked with several other areas within NIH including the Special Virus Cancer Program's Immunology-Epidemiology section and researchers from the Medicine Branch of NCI. He has served on the mathematics faculty of the NIH Federal City College Upward Mobility Program.

Dr. Brown's primary interest during the past year has been the evaluation of the risk of environmental carcinogens in which he has consulted with Gross at the F.D.A., Prival at the E.P.A., and Hoel at the N.I.E.H.S. He has also worked with Fernandez at the Census and Gonzalez at the N.C.H.S. on the problem of ethnic identification. He has provided statistical advice to Finegold at the V.A. Hospital in Los Angeles and Moore at the Anaerobic Lab in Blacksburg. His collaborative work with other investigators at the National Cancer Institute has included consultation with Dr. Radovich in the Breast Cancer Program Coordinating Branch and other members of the Biometry Branch, Dr. Godwin (prognostic factors in the survival of colon cancer), Dr. Williams (epidemiological analysis methods), Mr. Connelly (age distribution of cancer incidence), and Drs. Byar and Muenz (statistical methodology). He has also worked on aspects of the SEER Program and the Breast Cancer Epidemiology Program.

Serial No. Z01-CP-04250-10-B

Significance to the Program of the Institute:

The development of data resources and the provision of statistical consultation services are necessary contributions to the National Cancer Program.

Proposed Course of Project:

Planning efforts will be directed toward development of an optimum mixture of data resources. Consideration is being given to supplementing the SEER Program with a program of sampling from an enlarged geographic base in order to more effectively provide information needed for planning and evaluating the Cancer Control Program and the International Cancer Research Data Bank Program.

In order to more effectively carry out the expanded work scope of the Biometry Branch Program, the staff has been regrouped as follows:

Office of the Chief
Clinical & Diagnostic Trials Section
Mathematical Statistics & Applied Mathematics Section
Automated Data Processing Section
Demography Section
End Results Section
Field Liaison Section

The latter three sections are jointly responsible for implementing the SEER Program, under the direction of Dr. Cutler, Associate Branch Chief. The Automated Data Processing Section is intimately involved in helping the participants in the SEER Program organize or reorganize their data processing procedures to facilitate optimum utilization of the collected information.

Honors and Awards

Cutler, S.J.:

Member, American Joint Committee on Cancer Staging and End Results Reporting

General Secretary, International Association of Cancer Registries

Adjunct Professor, Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh

Haenszel, W.:

Member, American Cancer Society Advisory Committee on Intra-mural Research

Member, Editorial Advisory Board, Environ Res.

Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health

Member, American Association for Cancer Research Committee on Environmental Carcinogenesis

Member, Working Cadre of National Large Bowel Cancer Project

Member, Epidemiology Committee, Breast Cancer Task Force

Levin, .D.L.:

Member, Medical Oncology Trials Contract Review Committee, NCI

Member, Interagency Collaborative Group on Environmental Carcinogenesis, NCI

Murray, J.L.: Serial No. Z01-CP-04250-10-B

Member, Interagency Collaborative Group on Environmental Carcinogenesis, NCI

Publications

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Cutler, S.J. and Young, J.L.: Demographic Patterns of Cancer Incidence in the United States. Proceedings of Workshop on Persons at High Risk of Cancer, In press.

Haenszel, W.:

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Haenszel, W.: The United States Network of Cancer Registries. Springer-Press, Heidelberg, 50, "Recent Results in Cancer Research"

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Serial No. ZO1-CP-04254-02-B
1. Biometry Branch, OADFS&S
Division of Cancer Cause and Prevention
2. Bethesda, Maryland

NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Cancer in Defined Populations: Surveillance, Epidemiology, and End Results Reporting (SEER) Program

Previous Serial Number: Same

Principal Investigators: S.J.Cutler, H.Geller, M.H.Myers, J.L.Young,Jr.

Other Investigators: P.P.Connelly, B.F.Hankey, W.I.Lourie,Jr., J.L.Murray

Cooperating Units (SEER Program Participants):

California State Department of Health (NO1-CP-33353)
University of California, San Francisco (NO1-CP-43383)
Charity Hospital of Louisiana (NO1-CP-43262)
Connecticut State Department of Health (NO1-CP-43203)
Fred Hutchinson Cancer Research Center, Seattle (NO1-CP-33246)
Research Corp. of the University of Hawaii (NO1-CP-53511)
University of Iowa (NO1-CP-43200)
Michigan Cancer Foundation (NO1-CP-43264)
University of New Mexico (NO1-CP-33344)
University of Utah (NO1-CP-43382)
Yale University (NO1-CP-33235)

Man Years:

Total:	9
Professional:	5
Other:	4

Project Description

Objectives:

To provide descriptive data on cancer incidence and to investigate the variations in risk for a standard range of demographic variables including region, place of residence, race, nativity, economic status, and occupation; to identify promising leads and observational settings for epidemiological investigations to test specific hypotheses on etiological relationships; to evaluate patient survival for such characteristics as extent of disease at therapy, type of therapy, and over time.

Methods Employed: Serial No. Z01-CP-04254-02-B

The descriptive incidence data come from a group of population-based registries covering defined geographic areas which record for each cancer pertinent data on a standard list of characteristics including age, race, sex, place of residence, cancer site, histologic type, and periodically produce statistical reports and supply data on individual patients in accordance with a uniform code to the Biometry Branch. Some participants include epidemiologic research units which develop specific epidemiological hypotheses suggested by an analysis of morbidity and mortality data. They develop the study protocol and may pretest the instruments for data collection. From these preliminary investigations, proposals are generated which can be submitted for financial support either by government agencies through grants or contracts, or by nongovernmental institutions or agencies. The full investigation may be a cooperative activity involving a number of SEER programs. Active follow-up is accomplished either for the entire population, or for some subgeographic portion of the population for end results evaluation.

Major Findings:

Nine of the population-based registries were in operation by the end of 1973. Effort during the first few years will be toward insuring that there is comparability among participants in abstracting and coding of data submitted to NCI. This will be accomplished through field visits to assure there is complete reporting of all cases with active disease among residents of the area, and for auditing the quality of abstracted and coded data. Each participant will develop a package of computer editing for those portions of the code for which this is feasible.

The first submission of incidence data was received by NCI during January, 1975, for cases diagnosed in 1973. These data are being computer edited to test out the computer system and evaluate the data being submitted. Procedures will be developed for feedback to the participants and for correcting the submitted data.

An expanded code for Extent of Disease at time of therapy and a new code for Diagnostic Procedures used have been developed for cancers of the breast and colon/rectum. After field testing the codes, a series of workshops were conducted in order to train the participant staffs in the application of these new codes. All cases diagnosed during 1975 will be coded according to this new scheme.

Different techniques for operating the various phases of cancer registry operations, such as abstracting, coding, record matching for duplication, record storage, etc., are employed by SEER participants. This allows for evaluation and possible implementation to other registry operations. The San Francisco Bay Area registry has developed a computer program that matches new records with those already on file and makes a decision as to whether this is a new case, a previously reported case, or no computer decision can be made and the case must be reviewed. The potential for saving personnel time and improving searching accuracy is significant, especially for the large registries.

The Detroit SEER registry files are maintained on microfiche. All microfilming is done at the registry on automatic equipment. Hard copy can be obtained when needed. Filing space required for the microfiche is minimal, compared to the large area required for the original abstract records.

The New Mexico SEER registry has converted its records file to a color-coded system. Each digit 0-9 has a unique color. This system makes it easier to locate a record and to spot misfiled records.

Significance of the Program to the Institute:

Continuing information on incidence from representative groups of the U.S. population is required so that the nature and magnitude of the cancer problem including changes over time can be determined. Through close scrutiny of variations in cancer incidence with respect to geographic and demographic characteristics of the population and changes over time, specific etiologic hypotheses will emerge for testing which may lead to the identification of controllable risk factors. Continued recording of cases will also provide for evaluation of cancer control activities. Comparisons could be made for such factors as changes in stage of disease before and after the institution of the control program, compared to those detected through usual medical practice, etc.

Proposed Course of the Project:

1. The 1973 data will be edited and a methodology developed for correcting errors and adding cases to generate a final computer tape for 1973 incidence cases. Tabulations will be developed for those areas that have complete and accurate data.

2. The SEER programs that can generate follow-up data for cases accessed to their registries prior to the initiation of the SEER Program in 1973 will submit computer tapes on those cases. These data will be used to develop an end results report for distribution at the National Cancer Conference in 1976.

3. Expanded Extent of Disease and Diagnostic Procedures codes for cancers of the lung, stomach, cervix, corpus, and prostate will be field tested. Final codes will be developed and workshops conducted to train area staffs in the application of these new codes. The new codes will probably be implemented in January, 1976.

4. An audit of the abstracting and coding of a sample of 1973 cases, as well as an evaluation of completeness of reporting, will be done at each of the SEER programs. NCI staff and the facilities of the University of California Medical School in San Francisco will be used for this purpose, as well as for training new personnel and for the continuing education of existing SEER participant staffs.

5. An ad hoc committee has been selected to develop more detailed codes for therapy. These detailed codes will be site-specific and include all

modalities of therapy. The first sites of interest are breast and colon/rectum, for which there are expanded Extent of Disease and Diagnostic Procedures codes.

6. The 1973 incidence data will be reviewed by NCI and the epidemiologists at the SEER programs to identify possible areas of investigations. Study protocols will be developed and questionnaires field tested. It is hoped that by this time the mechanism for funding some of these projects through CREG will have been completed.

7. The 1974 incidence data will be accumulated and prepared for submission to NCI as soon after the end of the calendar year as possible.

8. It has been suggested that the SEER Program be reviewed by an outside committee. The Biometry and Epidemiology Contract Review Committee will do a first level review to determine how to effectuate a more detailed review if one appears to be desirable. It is proposed that this review encompass not only the objectives of the Program, but the procedures now used to meet these objectives. It is hoped the committee will develop recommendations that will reinforce the objectives and provide more effective ways of meeting them. In addition, it is hoped that a set of priorities can be specified so that modifications can be made more efficiently should there be budgetary restraints.

Serial No. Z01-CP-04257-18-B

1. Biometry Branch, OADFS&S
   DCCP

2. End Results Section

3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: STUDIES OF THERAPEUTIC END RESULTS AND PROGNOSTIC FACTORS

Previous Serial: Same

Principal Investigators: Sidney J. Cutler, Max H. Myers

Other Investigators: William I. Lourie Jr., Lillian M. Axtell, Herman Heise, Ardyce J. Asire, Evelyn M. Shambaugh, Benjamin F. Hanke, Anne Baranovsky, Philip Prorok, Debra T. Silverman

Cooperating Units: California Tumor Registry; Connecticut Tumor Registry; Charity Hospital of Louisiana; Indiana University Medica Center; State University of Iowa Hospital; University o Chicago Clinics; University of Michigan Hospital; University of Virginia Hospital; Saskatchewan Cancer Commission; New York Medical College.

Man Years:

Total:	15
Professional:	10
Other:	5

Project Description

Objectives:

To assist other Branches of the National Cancer Institute, other health agencies, and treatment centers in the development and maintenance of programs fo the collection and analysis of data on cancer in human populations with specia emphasis on the end results of treatment. To cooperate in international studies for the purpose of examining the relationship of etiologic and prognos tic factors in cancer. This includes consultation and advice on methodologica problems, development of improved statistical tools for the analysis of obser- vations on human populations, and active collaboration in the collection and analysis of data in a continuing collaborative program and in specific proje

Methods Employed: Serial No. Z01-CP-04257-18-B

1. The End Results Section staff provides advice on the development and application of uniform definitions and procedures to participating cancer registries, provides consultation on planning and implementing special studies, analyzes data submitted to the National Cancer Institute in accordance with a Uniform Code, and explores the use of more efficient data processing and statistical techniques to abstract the maximum amount of useful information from the collected data.

2. Standard statistical and actuarial techniques are utilized along with accepted principles for records management. Research is also done on the development of new statistical methodologies for analyzing patient survival data and, also, methodologies related to the design of cancer screening programs and the analysis of data collected by these programs.

Major Findings:

1. An analysis of data on 48 forms of cancer for patients diagnosed during the calendar period 1965-1969 indicated improvement in survival as compared to the calendar period 1950-1959 for some sites, but for other sites there has been little or no improvement. For patients with cancer of the larynx, prostate, testis, kidney, bladder, brain, thyroid, and with melanomas of the skin there were improvements. For patients with localized lung cancer and women with cancer of the breast there was moderate improvement. For Hodgkin's disease and multiple myeloma patients and particularly for children under 15 years of age with leukemia the improvement in survival has been substantial. The following table given 5 year relative survival rates by calendar period and stage (localized and all stages) for the aforementioned sites. In addition 1-year relative survival rates are shown for children under 15 years of age.

TABLE 1. 5-year Relative Survival Rates

		All Stages			Localized		
		1950 -59	1960 -64	1965 -69	1950 -59	1960 -64	1965 -69
Larynx	Male	56	54	62	74	72	80
	Female	57	52	59	71	67	73
Lung	Male	7	8	8	24	27	29
	Female	11	12	12	40	42	48
Breast	Female	60	62	64	83	84	84
Prostate	Male	47	52	56	61	65	68
Testis	Male	61	65	67	82	84	89
Kidney	Male	32	35	39	59	62	66
	Female	36	38	44	49	60	69
Bladder	Male	55	57	61	68	67	70
	Female	53	56	60	69	69	76
Melanoma of Skin	Male	51	52	61	70	74	75
	Female	60	70	73	79	82	85
Brain	Male	22	21	27	24	22	28
	Female	28	27	32	30	30	34
Thyroid	Male	70	77	78	88	92	99
	Female	83	86	87	95	96	97
Hodgkin's Disease	Male	31	38	52			
	Female	38	46	56			
Multiple Myeloma	Male	6	12	16			
	Female	8	10	16			

TABLE 2. 1-year Relative Survival Rate

		1950 -59	1960 -64	1965 -69	1970 -71
Acute Leukemia (under 15)	Male	26	40	60	76
	Female	21	41	62	71

2. Over 60,000 cases of histologically confirmed bowel cancers were analyzed by histologic type. Adenocarcinomas were the standard against which cancers of other diagnoses were compared. Colloid carcinomas occurred relatively frequently in blacks and in young persons, especially young men. In the left colon they had a worse prognosis than other cancers. Papillary carcinomas had a better than average prognosis while polypoid carcinomas had the best prognosis of any type of carcinoma. Adenosquamous and squamous cell carcinomas in the rectum closely resembled anal cancers; elsewhere in the bowel they behaved like aggressive adenocarcinoma. Malignant carcinoids of the appendix resembled benign carcinoids of that organ; elsewhere they were quite aggressive. Rectal carcinoids were unusually common in blacks. Grade appeared to be an independent prognostic parameter, although low grade of malignancy also implied an early stage. The differences in behavior by histology point up the importance of specificity of histologic diagnosis for cancers of the bowel.

3. In order to evaluate the effects on survival of several variables for cancer of the colon and rectum, data on 11,374 cases were analyzed. Cases were all histologically confirmed as malignant and were restricted to adenocarcinomas without designation of special sub-types (e.g., colloid or papillary). Dates of diagnosis were 1965-1971. In comparing the survival of selected patient groups, a methodological technique was used that permitted adjustment for relevant confounding variables. Extent of disease was the variable with the biggest impact on survival. Other factors influencing survival were primary site of the neoplasm, presence of local vessel invasion, histologic grade, sex, and age of the patient. Results were generally comparable with other series; the only difference was the favorable survival of young patients.

4. In cooperation with the Epidemiology Branch an analysis of 644 children under the age of 15 with Wilms' tumor was carried out. It is known that mortality from Wilms' tumor in the U.S. has decreased by about a third between 1940 and 1969 while the annual incidence rate has remained relatively constant. These two observations would be consistent with an improvement in patient survival and it has been suggested in the literature that improved survival is due to earlier diagnosis. A review of our data, however, revealed that while the three year survival rate has doubled over the period 1940 to 1964 the mean age at diagnosis and the percent of cases diagnosed as localized both remained relatively constant. There were substantial changes in treatment as an increasing percentage of patients received surgery and/or radiotherapy within four months of diagnosis and chemotherapy, little used before 1960, was administered to 83% of cases by the period 1965 to 1969.

5. In cooperation with the American Cancer Society a Professional Education Publication on childhood cancers was published. Utilizing the Third National Cancer Survey incidence rates and mortality rates for the United States in 1969, the following estimates were made for the number of new cancer cases and the number of cancer deaths in children under 15 in the United States.

	New Cases	Deaths
All Sites	6500	3600
Leukemia	2150	1650
Brain and Central Nervous System	1350	930
Lymphoma	650	300
Kidney	400	180

The five year relative survival rates for children diagnosed 1955-64 ranged from a low of 3 percent for leukemia to 98 percent for thyroid gland. The five year relative survival rates for some other sites are; central nervous system-36 percent, kidney-41 percent, lymphoma-29 percent, and for all forms of cancer-30 percent.

6. Our staff also cooperated in the writing of a publication intended to serve as a reference volume for physicians, medical students, public health workers, and other individuals concerned with the course of cancer in individual patients or with its effect on various population groups. The purpose of the report is to present information on some of the measurable aspects of cancer. The material covers variations and trends in cancer incidence and mortality, some aspects of diagnosis and treatment, the epidemiologic characteristics of the more important cancer sites, survival rates for diagnosed cancer cases, and prospects for future progress.

7. Work has continued on the development of a comprehensive theory for the design and evaluation of screening programs for the early detection of disease. Effort has concentrated on extending the theory to the case of repetitive (but not necessarily periodic) screening of a population to effect the early detection of a progressive chronic disease. In this setting, the distribution theory of bounded recurrence times of the stochastic model representing the natural history of the disease serves as a foundation for the methodology. A new method for deriving this distribution theory which encompasses nonstationary as well as stationary models has been developed. Theoretical expressions for the distribution and properties of the lead time gained by screening have been derived. These expressions are functions of the intervals between screens, the number of screens, and other parameters which are important in the design of a screening program. With respect to the evaluation of a screening program, expressions for estimating the mean lead time and the probability of detecting preclinical disease have been obtained. Numerical estimates of the mean lead time have been calculated using data from a repetitive screening project for the early detection of breast cancer conducted by the Health Insurance Plan of New York in New York City.

8. Work has continued on the utilization of various types of mathematical models for analyzing response-time data (survival data). The most recent model to be considered incorporates the geometric distribution and the multi-variate logistic function. The logistic function may include both regressor

variables and some function of time allowing the conditional probability of dying (surviving) within a time interval to vary over time. The model has been used to analyze data on melanoma patients where the regressor variables were depth of penetration of the primary, ulceration of the primary, and clinical assessment of regional lymph node involvement. All three variables were found to have an important effect on survival. The end point used in this analysis was death with melanoma present. Such a model permits more accurate estimation of survival rates when the change over time in the logit of the conditional probability of dying is similar in the various patient subgroups specified by the regressor variables.

Significance to the Program of the Institute:

Many of the benefits of the consultation program are long range in nature and consist of setting up record systems and study situations which enlarge the potential study resources of NCI and related organizations. The research objectives of the Institute are promoted by continued work on the integration of various study techniques, e.g., retrospective and prospective studies, analyses of mortality data and morbidity surveys, epidemiologic investigations and cancer registries. An example of the interaction between epidemiologic and end-results investigations are studies of prognostic factors and the role of these factors in cancer etiology.

Proposed course of the project:

1. This project is part of the Collaborative Program for Cancer Surveillance, Epidemiology and End Results Reporting (SEER Program) being developed within the Biometry Branch. Emphasis will continue to be placed on obtaining and reporting data on cancer therapy and associated survival patterns. The SEER program has currently been expanded to include registries covering the incidence of cancer in defined populations, therefore, studies of the association of end results and the incidence of cancer will be possible for defined populations.

2. Activities in the development of special studies on prognostic factors will continue. In some cases these studies will be in collaboration with the American Joint Committee for Cancer Staging and End Results Reporting, in other cases with cancer specialty centers such as Memorial Hospital (N.Y.C.). With the development of the Resource for Cancer Epidemiology associated with the California Tumor Registry and the Research Unit in Connecticut, the potential for an enlarged special study program will be greatly increased.

3. An expansion of project activities is expected in the area of international collaboration. A pilot study of patient survival and the distribution of cancer patients by extent of disease is underway among countries with varying levels of breast and colon cancer incidence. This study should reveal the feasibility of conducting detailed studies to determine the relationship between factors associated with levels of incidence, the degree of cancer spread at diagnosis, and survival subsequent to diagnosis. Representatives from Finland, Norway, Birmingham, England, and the State of Connecticut have indicated an interest in this type of study and have agreed to participate in an assessment phase of a study of breast and colon cancer.

Publications

Myers, M.H., Young, J.L., Silverberg, E. and Heise, H.W.: Cancer Incidence, Survival and Mortality for Children Under 15 Years of Age. American Cancer Society Publication No. 3022 PE. New York, American Cancer Society, 1974, 18 pp.

Axtell, L.M., Myers, M.H., Christine, B. and Linden, G.: Trends in Survival and Classification for Patients With Leukemia Diagnosed 1940-69. JNCI 53: No. 2, 375-377, August 1974.

Everson, B.B., Fraumeni,Jr., J.F. and Myers, M.H.: Improved Survival in Wilms' Tumor. The Lancet: 1290-1291, June 1974.

Berg, J.W., Godwin, J.D. II: The Epidemiologic Pathology of Carcinomas of the Large Bowel. J. of Surg. Oncol.: 381-400, 1974.

Axtell, L.M., Myers, M.H. and Shambaugh, E.M.: Treatment and Survival Patterns for Black and White Patients Diagnosed 1955 through 1974. DHEW Publication No. (NIH)-75-767, 1975.

Serial No. ZO1-CP-04258-08-B
1. Biometry Branch, OADFS&S
Division of Cancer Cause and Prevention
2. Demography Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of Cancer Incidence and Related Etiologic Factors

Previous Serial Number: Same

Principal Investigator: William Haenszel, Haitung King, and John Young

Other Investigators: Roger Connelly, Susan Devesa, Frances Locke, Constance Percy, Joseph Scotto, Roger Williams

Cooperating Units: Aichi Cancer Institute, Nagoys, Japan
Kuakini Hospital, Honoulul
Cancer Registry of Norway
Universidad del Valle, Cali, Columbia
Framingham Field Unit, NHLI
Breast Cancer Demonstration Project, DCCR, NCI
Temple University School of Medicine

Man Years:
Total: 12
Professional: 7
Other: 5

Project Description

Objectives:

To describe cancer morbidity and mortality in human populations; to seek out and examine significant differences between persons who develop cancer and those who do not; to search for clues to causative or promoting factors of cancer by comparisons between high- and low-risk populations; an example of the latter being certain migrant groups in the U.S. useful for the study of host and environmental effects in specific cancer sites; to relate epidemiologic features of cancer to survival rates; to identify personal and environmental factors of potential significance in the prevention, control, early diagnosis, or successful treatment of cancer.

Method Employed:

These studies require an extremely wide variety of methods of data collection and analysis, and in many cases new methods must be developed. Where appropriate, these are described in the following sections.

The Migrant Studies Program currently consists of four components: Norwegian, Japanese, Colombian and Polish (sponsored by PL-480 funds in Poland). Observations have established the nature of the changes in site-specific cancer-risks in these migrant populations. In all populations efforts are directed toward obtaining comparative epidemiologic and supporting pathological data while exploiting the special capabilities of each collaborator and to take advantage of the experiments of nature represented by migration. The major efforts have been directed to stomach, large bowel and prostate.

Stomach. The contributions of migrant studies to the development of stomach cancer epidemiology has recently been reviewed and the highlights of events of the past 10 years are summarized below:

a. The persistence in the U.S. of high stomach cancer risks among migrants from high-risk countries pointed to factors presented in the environment of the home country.

b. Muñoz, Correa and others typed stomach cancers in Latin-American populations at high- and low-risk to the disease using the criteria of Jarvi and Lauren and reported the intestinal type to constitute a higher proportion of cases in high-risk areas. From this they inferred the intestinal type to be an "epidemic" component of stomach cancer responsible for most of the difference between high- and low-risk areas.

c. The cancer registry in Cali, Colombia, described marked variation in stomach cancer risk among residents of that city by birthplace, the marked excess of cases occurring among migrants from the department of Nariño. The next critical step in Cali was to elaborate the registry findings by autopsy studies, which showed intestinal metaplasia to be more prevalent among the migrants from Nariño.

d. The case-control study of Hawaiian Japanese revealed that migrants from the Japanese prefectures with highest stomach cancer risks continued to experience an excess risk in Hawaii, but this effect did not persist among their Nisei offspring. Lower risks were suggested for the Nisei, but not Issei, who had adopted Western-style diets.

e. The companion case-control studies conducted in Japan (Hiroshima and Miyagi prefectures) did not reproduce the associations with the pickled vegetables and salted/dried fish reported for the Hawaiian Japanese. Characterization of the farm population as the group at highese risk to stomach cancer was the important finding from work in Japan.

f. The pathology studies on stomach cancer cases in Miyagi prefecture and Hawaii elaborated earlier distinctions for intestinal and diffuse types. Since tumor registries provide incidence data for both populations, type- and age-specific incidence rates could be

estimated. The incidence for diffuse carcinomas was substantially the same in both localities, but the incidence for intestinal, mixed and other types was markedly lower in Hawaii.

g. The findings on intestinal metaplasia suggested the transformation of the epidemiology of stomach cancer into the epidemiology of intestinal metaplasia to be a reasonable line of attack. A cohort of Japanese males in Hawaii assembled and under surveillance by the National Heart and Lung Institute was chosen for this purpose. A variety of physiological, anthropometric, blood chemistry and dietary observations had already been assembled and another round of examinations collected data relevant to gastro-intestinal cancers. Tests for gastrin bioassay, parietal cell antibodies and pepsinogen I and II were included to provide information on presence and degree of intestinalization of the gastric mucosa.

h. More promising opportunities for gastroscopic studies of intestinal metaplasia have been presented in Colombia. A preliminary assessment of hospital admissions and discharges confirmed the clinical impressions of local physicians that stomach cancer risks were not uniformly distributed throughout Nariño, but instead were concentrated in certain localities and this information was taken into account in the conduct of field work. The primary objective of the examination of a population of apparently well individuals by gastroscopy was to determine the prevalence of intestinal metaplasia and/or chronic atrophic gastritis, but individuals examined were also questioned with respect to residence history, food history and sources or water supply. The distribution of intestinal metaplasia and chronic atrophic gastritis by place of birth agreed well with the previously noted clustering of stomach cancer cases. Persistence of the high prevalence of intestinal metaplasia among individuals born in the high-risk communities, but later moving to low-risk areas (by Nariño standards), reinforced the pervasive theme on importance of exposures in early life for this disease. The presence of wells with nitrate-rich water in several of the high-risk (for both stomach cancer and intestinal metaplasia) communities in Nariño is provocative in view of other evidence tentatively linking nitrates with stomach cancer.

The great virtue of the migrant population approach has been its ability to correlate observations over a wider spectrum of environmental exposures in home and host populations, which have clarified the following features of stomach cancer: The critical role of exposures in early life in determining the level of risk; the important distinction between intestinal and diffuse types of the disease for descriptive and analytical epidemiology, this does not imply that the final word has been said on the meaning and significance of the type distinctions; documentation of intestinal metaplasia as a candidate precursor lesion; and detection of associations with certain classes of foods that had escaped attention in studies confined to sedentes.

Colon. A case-control study in the Hawaiian Japanese--a population undergoing displacement from low to high colon cancer risk and in varying stages of transition from Japanese- to Western-style diet--has uncovered leads that are being investigated in animal models, a program that is being supported by the Carcinogeneseis Area, NCI, and the National Large Bowel Cancer Project. There is general agreement that a new round of case-control studies of diet should await the findings of and take note of these results of animal work.

Additional studies of the distribution of intestinal polyps in high- and low-risk populations for large bowel cancer in New Orleans, Honolulu (Japanese) and Japan expanded on the findings for cancer of the colon and rectum and adenomatous polyps published by Haenszel and Correa. Stemmermann and Yatani (Cancer) have reported the distribution of both the adenomatous and hyperplastic polyps among Hawaiian Japanese to parallel closely the New Orleans experience, while newly-published data from Japan portray a low prevalence of both adenomatous and hyperplastic polyps, which parallel the results from Colombia. There can be no doubt that among Japanese migrants a profound change in the number and the anatomical distribtuion of adenomatous and hyperplastic polyps has accompanied the marked risk in colon cancer risks in the migrant Japanese. Polyp studies in other populations at low-risk to large bowel cancer (Costa Rica and Sao Paulo area of Brazil) are nearing completion and will provide useful documentation on precursor conditions.

Prostate. The work of Akazaki (JNCI 50: 1137-1144, 1973) offers the possibility of resolving anomalous observations on the prevalence of occult prostatic carcinoma and clinically active disease for Japanese populations at home and abroad. Akazaki distinguished between two forms of occult disease -- proliferative, non-proliferative -- and was able to demonstrate a rise in prevalence of the proliferative type among Hawaiian-Japanese consistent with their increased incidence of clinically active disease. An effect limited to a subset of more aggressive appearing occult lesions suggests a role for some unidentified promoting factor. These findings have led to the design of a case-control study in Hawaii and Japan, supplemented by collection of additional data on the cohort of Japanese males being followed in Hawaii by investigators associated with the ongoing contract-supported program (Drs. Rhoads and Aoki). A revised proposal has been submitted to the Prostate Cancer Task Force for consideration.

The Framingham Cancer Study is a joint project of NCI and NHLI. From the cohort of 5,209 patients followed for 22 years in the Framingham Heart Study all patients with cancer are being identified. Pertinent information (date of cancer diagnosis, type and site from pathology reports, etc.) is obtained for each cancer patient. When complete this roster of cancer patients will be compared to the noncancer patients in the cohort with respect to many interesting variables followed during the 22 year heart study. Variables include historical items (alcohol and coffee consumption, smoking, education, birth control pills, number of offspring, etc.); physical findings (abnormal lymph nodes, obesity, eye color, etc.); laboratory findings (serum cholesterol, blood type, chest x-ray, etc.); and diagnosis (rheumatoid arthritis, diabetes, hypertension, etc.).

The overall purposes are to:

(a) test known hypotheses (e.g., cholesterol and colon cancer)

(b) find possible new associations to form new hypotheses and stimulate other research (e.g., drugs vs. cancer types)

(c) in selected cancer patients, analyze frozen sera saved from before their diagnosis to investigate the time course to tumor associated antigens.

Development of the National Cancer Program has reinforced the need for a U.S. network of cancer registries for information required for planning and administration and to supply continuing data on incidence and treatment end results. Much time has been spent in the past year on implementation of the recommendations of the Advisory Committee on Population-based Epidemiology Research Centers.

The Preliminary Case Control Study of Reserpine and Breast Cancer is a joint project of NCI, NHLI and ACS. A report in Lancet (September 21, 1974) suggested an association between mammary cancer and this drug commonly used to treat hypertension. The purpose of this study is to gather further data to test for this association as requested by the directors of NCI, NHLI, and FDA.

From 90,000 women screened for breast cancer at 27 Breast Cancer Demonstration Project Centers, over 400 new cases have been diagnosed. These breast cancer patients and 1,200 controls (matched for location, age, race, and screening date) will be asked by mailed questionnaire whether they have had hypertension and used any drugs to treat hypertension. They will be asked for drug names, duration of use, prescribing physician's name and address. For those taking antihypertensives the prescribing physician(s) listed by the patient will be asked by mailed questionnaire to verify drug names and duration of use. Non-responding patients or physicians will be contacted by phone or visit of American Cancer Society volunteers.

Data will also be available on the patients for potentially confounding variables such as socioeconomic status, parity, height, weight, etc. to allow for correction during analysis. The project is expected to take 6-9 months to complete.

Analysis of noncancerous disorders among the Chinese. A comprehensive analysis has been completed of epidemiologic data on the population in the United States and Asia over the past two decades. One noticeable feature is the rise of coronary heart disease in relation to transition of cancer and vascular lesions. The elevation of coronary heart disease among the U.S. Chinese, as compared to the low level for their Asian counterparts, parallels that for colonic cancer but contradicts that for cancer of the stomach. The same may be said of the rise in vascular lesions in relation to colonic and stomach malignancies. This is in keeping with the findings on the U.S. Japanese and other international comparisons.

Review of current research in cancer epidemiology in China. Two outstanding features may be mentioned. One is the possible role of nitrosamines in the development of esophageal cancer. Another is an association between epithelial hyperplasia and malignant changes of esophageal mucus. Opportunities for collaborative research between this country and China in the future are indicated.

Cancer in the clergy. The results of further investigation into clerical mortality patterns of the United Presbyterian Church, U.S.A. and the Lutheran Church-Missouri Synod are consistent with those previously reported for United Lutheran and Episcopalian clergy. For example, despite the much favorable overall mortality among the four cleric groups, compared to the general population, prostatic and intestinal cancers, leukemia, and lymphoma are among the causes with less favorable risk. However, lung cancer has a relatively low mortality.

NCI has developed (see NCI-4254) a coordinated program of cancer Surveillance, descriptive Epidemiology and End Results evaluation (SEER Program). The output of this multi-purpose program will be the basis for:

(a) prompt identification of secular trends in specific cancers;

(b) identification of variation in disease incidence that may lead to etiological hypotheses;

(c) assessment of the efficiency and quality of the medical care system;

(d) studies by which primary and secondary preventive measures can be accurately measured.

The descriptive incidence data from population-based registries will lay the groundwork for investigations focused on etiology, either in the form of analytic epidemiological studies or animal experiments.

NCI research and program goals do not presently require, or even suggest an ultimate need for, the establishment of a large scale program, national in scope, of cancer registration. However, the incidence and surveillance objectives enumerated require both:

(a) Population-based cancer registries. These will provide the bulk of the descriptive incidence and end results data.

(b) Population-based Cancer Study Centers. These will augment the conventional registry effort with diagnostic, therapeutic, epidemiologic, statistical, laboratory, and other skills on which valid data on incidence and end results depend. Some Population-based Cancer Study centers may be part of a Comprehensive Cancer Research Center.

The coordination of work in cancer epidemiology to be carried out by population-based cancer study centers and comprehensive cancer research centers does present a completely new dimension for a national program.

The existing model for data handling represented by the End Results Group is not relevant, since each center will be engaged in a broad panorama of multidisciplinary research and training activities not amenable to centralized direction and control. The mechanism to facilitate exchange of information among the research centers and to enable them to develop programs compatible with broad national goals will be a committee which will include the directors of the several research centers as members. The second formal meeting of this committee was held in Detroit, Michigan, September 19-20, 1974.

Incidence data for the year 1973 were submitted to NCI by eight of the nine SEER program participants in January, 1975. Data from the Third National Cancer Survey (TNCS) were used to estimate the number of cases which should have been reported. There was some variation from expectation and investigation is being made to determine whether certain registries may be missing incident cases of cancer. Population data for the SEER areas are being amassed so that 1973 incidence rates can be compared to those obtained in the TNCS 1969-71. However, there are no plans to publish 1973 incidence data before further quality control checks on the data have been completed.

Ultraviolet Radiation Study. Together with the Department of Transportation, Temple University and the National Oceanographic and Aeronautics Administration (NOAA), the National Cancer Institute is in the process of collecting and analyzing new data on ultraviolet radiation. The Department of Transportation was initially concerned over the depletion of atmospheric ozone caused by continuous flying of SST's (supersonic transport jets). Recent results from the Third National Cancer Survey's skin cancer study showed an inverse relationship in incidence with latitude, but measures of the amounts of carciongenic ultraviolet remained unknown. During the past year Robertson-Berger meters, designed to measure sunlight ultraviolet reaching the earth's surface, were stationed in key locations throughout the United States and other parts of the world, including several areas where epidemiologic data have been collected by the TNCS or will be collected in the near future by the SEER group.

After the completion of one year's recordings of SUV units at each location, analyses will focus on latitudinal and temporal patterns (e.g., variations by time of day, daily, monthly and annual counts), and the effects of cloud cover and other weather conditions on meter readings. The association of ozone level, SUV, and skin cancer incidence will be evaluated at each location where data are available.

Significance to the Program of the Institute:

This program helps establish baselines for cancer mortality and morbidity in various populations from which changes can be measured, ascertains trends in risks which may reflect the action of various etiologic agents, identifies high- and low-risk population groups which can be studied and compared, and provides observations on human populations useful for correlation with laboratory studies. Ad hoc investigations are undertaken because of unusual or unexpected findings from the routine monitoring of incidence and mortality rates.

The migrant population studies represent a major biometric effort of the Institute. If these studies elucidate the environmental facotrs involved in the incidence of certain forms of cancer and indicate those which are predominantly environmental and those which are predominantly genetic, it may be possible to apply these findings to the United States population for purposes of cancer prevention and cancer control.

Proposed Course of the Project:

The migrant studies will emphasize analysis of data bearing on the pathogenesis of the major sites under study, particularly large bowel. The findings from the case-control studies of gastrointestinal cancers and diet will be used in developing the protocols for the ongoing cohort study of Hawaii Japanese males and the extension to cohorts in Japan. Field studies using the gastrocamera and other screening techniques of the population at very high risk for stomach cancer in one department of Colombia are receiving high priority. Greater efforts in the synthesis of data in hand and being collected are required and this will require closer collaboration with the principal investigators for the several related programs being supported by research contracts.

Incidence data for the year 1974 will be submitted to NCI by SEER program participants in January, 1976. These data will be analyzed by staff to investigate both geographic differences and time trends in incidence by site and type. Comparison with data from the Third National Cancer Survey will be possible and in addition to serving the purpose of formulating time trends, such comparisons will serve as further quality control checks for programs in the SEER Program.

The immediate course of the other projects is not predictable, since many are undertaken as the need becomes apparent and as staff time is available. A number of studies are in various phases of completion, and the items listed below represent long-term efforts and shortage of staff resources will delay completion until at least the following year.

Critical review of epidemiologic research in China will be made periodically to provide suggestive clues for potential collaborative studies and guidelines for possible field trips.

Plans for a study of the use of herbs among the Chinese in this country and other areas will be completed.

Studies of clerical mortality patterns will continue in order to establish more firm evidence on cancer risk in this profession. The final results would be useful for initiation of similar studies on cancer in other learned professions.

Ten years of follow-up data are available for 3,500 polyp-free patients, and fifteen years of follow-up data have been collected on an additional 500 patients, all examined in the mid-1950's at Lahey Clinic, Boston. The Lahey Clinic has collected fifteen years of follow-up on 300 patients examined during the same time period found to have rectal or colonic polyps at

examination. Both the polyp-bearing and polyp-free groups experienced fewer than expected colon and rectal cancers during the first ten years post examination. However, the polyp-bearing group showed a far greater than expected number of these cancers during years eleven to fifteen post examination, so that it is of extreme importance to complete the fifteen-year follow-up for the entire polyp-free series to permit accurate comparisons between the two groups. This will be undertaken if staff is available.

Honors and Awards:

King, Haitung:

Clinical Professor of Community Medicine and International Health, Professoral Lecturer and Research Associate in Demography, Georgetown University.

Reviewer for Social Science and Medicine.

Paper entitled "Selected Aspects of Cancer Epidemiology in the People's Republic of China" presented at the 11th International Cancer Congress, Florence, Italy, October 19-26, 1974.

Paper entitled "Needed Areas of Epidemiologic Research on the Chinese" presented at the annual meeting of the Society for Epidemiologic Research, Albany, New York, June 20-21, 1974.

Paper entitled "Mortality Among Foreign and Native-Born Chinese in the United States" presented at the annual meeting of the American PUblic Health Association, New Orleans, Louisiana, October 21-24, 1974.

Invited paper entitled "Current Research in Cancer Epidemiology in the People's Republic of China" presented at the Third World Symposium on Acupuncture and Chinese Medicine, New York City, March 21-23, 1975.

Percy, Constance:

Member, Morphology Committee for the Development of the World Health Organization International Classification of Diseases - Oncology Supplement (ICD-O).

Member, Indexing Committee for the Neoplasm Chapter of the 9th Revision of the World Health Organization International Classification of Diseases.

Member, Subcommittee for Ground Rules of Coding, Indexing Committee, 9th Revision of the World Helath Organizations International Classification of Diseases.

Invited paper on "Apparent Changes in Cancer Mortality, 1968. A study of the effects of the introduction of the Eighth Revision International Classification of Diseases." presented at the XIth International Cancer Congress, Florence, Italy, October, 1974.

Scotto, Joseph: Serial No. Z01-CP-04258-08-B

Reviewer for Science, Inquiry, Journal of the National Cancer Institute.

Consultant: Climatic Impact Assessment Program - Subcommittee of Effects of the SST.

Invited lecture on "Epidemiological Survey of Ultraviolet Radiation" presented at the 15th Meeting of the Interagency Collaborative Group on Environmental Carcinogenesis, Bethesda, Maryland, February, 1975.

Publications:

Haenszel, William: Migrant studies: contributions to cancer control and epidemiologic research (submitted - to be published in a monograph entitled Persons at High-Risk to Cancer: An Approach to Cancer Eiology and Control).

Haenszel, William; Correa, Peylao; Cuello, Carlos: Social class differences in large bowel cancer in Cali, Colombia (in press in JNCI).

Haenszel, William: International collaborative studies of cancer in man: role of the National Cancer Institute. National Cancer Institute Monograph 40: 75-81, 1974.

King, Haitung: Cancer Control in the People's Republic of China. In proceedings of the Second International Symposium on Cancer Detection and Prevention, Bologna, Italy, April 9-12, 1973, pp. 551-559. Editor: Cesare Maitoni-Excepta Medica, Amsterdam, 1974.

King, Haitung: Further Inquiries into Protestant Clerical Mortality Patterns, Biosocial Science, in press.

King, Haitung: Selected Epidemiological Aspects of Major Diseases and Causes of Deaths Among the Chinese in Asia and Other Areas. In Proceedings of the Conference on Comparative Studies of Modern and Traditional Medicine in Chinese Societies, Seattle, Washington, February 4-7, 1974. NIH Fogarty International Center, in press.

King, Haitung: Review on Ethnic Groups of America: Their Morbidity, Mortality, and Behavior Patterns, Volume 1, the Jews. By Ailon Shiloh and Ida Cohen Selavan (eds.). C.C. Thomas, 1973. Social Science and Medicine, in press.

Percy, C.L., Garfinkle, L., Krueger, D., and Dolman, A.: Apparent Changes in Cancer Mortality in 1968 as a Result of the Introduction of the 8th Revision, International Classification of Diseases. Public Health Reports 89:418-428, October, 1974.

Prager, E.D., Scointon, N.W., Young, J.L., and Veidenheimer, M.C.: Follow-up Study of Patients with Benign Mucosal Polyps Discovered by Proctosigmoidoscopy. Diseases of Colon and Rectum 17:322-324, June, 1974.

Serial No. ZO1-CP-04260-15-B

1. Biometry Branch, OADFS&S
   Division of Cancer Cause and Prevention
2. Clinical and Diagnostic Trials Section
3. Bethesda, Maryland

NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Consultation on Clinical Trials

Previous Serial Number: Same

Principal Investigator: David P. Byar

Other Investigators: Mitchell Gail, Donald K. Corle, James A. Koziol, Sylvan B. Green, and Anna J. Watkins

Cooperating Units: V. A. Cooperative Urological Research Group (26 hospitals)

Man Years:

Professional:	5
Other:	1
Total:	6

Project Description

Objectives:

To provide consultative services in statistical and epidemiological methodology necessary for the various programs sponsored by the Veterans Administration Cooperative Urological Research Group. This includes assistance in the design, interpretation and evaluation of the clinical trials conducted by the Veterans Administration Cooperative Urological Research Group, and provision for studies operating concurrently with the clinical trials such as epidemiological studies.

Methods Employed:

Standard methods of biometry, statistics, probability, and epidemiology with necessary modification as required by the particular problem. New techniques are developed by the personnel working with the Section to handle specific problems.

Major Findings:

This Section assists the investigators in developing and evaluating methods of treatment for cancers of the male genitourinary tract. Such assistance is generally acknowledged in the publication of findings of the medical investigators. New statistical techniques are published independently.

The Section is currently involved in four active clinical trials. Two new trials in cancer of the prostate will be begun in April 1975 (see below). Two trials reported in the last annual report, the Focal Carcinoma Study and the Urinary Bladder Study I, have been completed and the results have been published. Patients are still being followed in the Focal Carcinoma Study, however, and this trial serves as a useful comparison for the ongoing main prostate studies. The four main trials are:

(a) Main Prostate I, which compares the effect of two treatments (prostatectomy alone, and prostatectomy with estrogen) for Stages I-II, and of four treatments (estrogen, orchiectomy, estrogen plus orchiectomy, and placebo control) for Stages III-IV. The intake of new patients into this study was terminated on March 31, 1967. A total of 2,313 patients had been entered by that date.

(b) Main Prostate II, which also compares the effect of two treatments (prostatectomy alone and placebo control) for Stages I-II and of four treatments (three levels of estrogen and placebo control) for Stages III-IV. This study is designed as a sequel to Main Prostate I. Intake of new patients was terminated in June 1969 after 561 patients had been entered in the study.

(c) Main Prostate III, which compares placebo versus prostatectomy for operable Stage I-II patients. Inoperable Stage I-II patients are randomly assigned to placebo or 1.0 mg. Diethylstilbestrol (DES). All eligible Stage III-IV patients are randomized to one of four treatments: 1.0 mg. DES, 30 mg. Provera, 2.5 mg. Premarin, or 30 mg. Provera plus 1.0 mg. DES. As of September 13, 1974, 1254 patients had been entered in this study.

(d) Urinary Bladder II, which compares transurethral surgery followed by oral placebo, oral pyridoxine, or topical instillation of thio-TEPA in treating Stage I tumors of the bladder. Intake of new patients into this study was begun in November 1971. As of September 13, 1974, 107 patients had been entered in this study.

The data from two trials -- Main Prostate III and Urinary Bladder II -- are not yet sufficient for detailed study.

The results of Main Prostate I have already caused widespread changes in the general treatment of prostatic cancer, because of the finding that the standard estrogen treatment for prostatic cancer carries a very great risk of death from cardiovascular disease. In addition, the clinical benefits with respect to the cancer are smaller than was generally believed. Overall

mortality rates in Stages I, II, and III, and in "early" Stage IV are substantially higher in estrogen-treated patients than in those not receiving estrogens. This hazard was not recognized before the results of this study were available.

During the past year we have continued to explore the usefulness of an exponential survival model in identifying variables of prognostic significance in cancer of the prostate. The first article on this subject, based on the data from the Study I for Stages III and IV patients, was published February 1974. Variables identified as important for predicting the course of prostatic cancer included the acid phosphatase, the presence or absence of bony metastasis, the age of the patient, the presence of the ureteral dilation at the time of diagnosis, the level of hemoglobin, performance status, and presence or absence of pain due to cancer. In the last fiscal year two additional articles were published on this subject as a result of the collaboration with investigators at Yale University described in last year's annual report. The first of these concerned selection of variables in an exponential survival model using a pseudo-likelihood step-down procedure. The second paper compared the analysis of data using this procedure with that obtained by more ordinary statistical methods such as cross-tabulations and survival curves and illustrated the convenience and utility of this model.

The results from Main Prostate II indicated (1) that there is no evidence thus far to show that 5.0 mg. DES is superior to 1.0 mg. in controlling the cancer in either Stage III or IV, (2) that in Stage III there are substantially fewer heart and cardiovascular deaths on 1.0 mg. DES compared to the 5.0 mg. dose, (3) that in Stage IV both the 1.0 mg. and 5.0 mg. doses are more effective than the 0.2 mg. dose in controlling the cancer, and (4) that in Stage III the superiority of placebo over the 5.0 mg. dose (and to a lesser extent over the other two doses of DES) is accounted for by the excess of deaths from non-cancer causes in the estrogen-treated patients. Taking all these rather complex relationships into account we see no justification for the continued use of 5.0 mg. DES in either Stage III or IV.

In the past year further analysis of the data from Study II has focused on the prognostic significance of biochemical measurements performed at the time of diagnosis and the response of these measurements to treatment. We have found that the initial values of 17-hydroxycorticoids provide useful information about prognosis. A paper on this subject was published in the New England Journal of Medicine indicating that high initial values are associated with a poorer prognosis. Two other papers have been completed in this fiscal year, the first devoted to plasminogen and fibrinogen, the second to cholesterol and triglyceride. These have recently been submitted to the British Medical Journal. Both the pre-treatment plasminogen and fibrinogen levels were found to contain useful prognostic information; specifically high initial fibrinogen levels and low initial plasminogen levels were associated with poorer prognosis. Cholesterol was not found to be related to prognosis in patients with prostatic cancer, however, analysis of the data for triglyceride revealed that a higher

risk of death was associated with values in the middle of the range. This finding, although confirmed by a variety of statistical tests, is in disagreement with the previously published reports and suggests that lipid metabolism in patients with prostatic cancer may be quite different from that in patients without cancer.

Another major area of interest in the analysis of data from Study II has been the application of the exponential survival model in an effort to determine if there is a treatment of choice for individual patients based on their pre-treatment characteristics. We were able to identify certain subsets of patients who would have benefitted from initial treatment with estrogen and another group of patients who would have clearly benefitted from initial treatment with placebo. Thus, in this study, no one treatment was best for all patients, but rather the patients' pre-treatment characteristics determined their treatment of choice. The important aspect of this work is that a statistical methodology has been developed for identifying such treatment-covariate interactions. This work was presented by invitation at the American Statistical Association meeting in August in a symposium on the analysis of survival data from heterogeneous populations.

It is expected that acquisition of patients from Main Prostate III will be terminated in March of 1975. Thus far the results of this study have revealed no advantage of Provera or Premarin over the 1.0 mg. dose of DES for patients in stages III and IV. The frequency of cardiovascular deaths in patients treated with Premarin has been no different from that observed on the 1.0 mg. dose of DES. Thus it still appears that 1.0 mg. DES daily is the best treatment for symptomatic prostatic carcinoma.

As yet there is insufficient data in the Bladder Study II to draw any conclusions. However it is noteworthy that trends observed to date indicate that the therapeutic effect of pyridoxine (Vitamin B6) lies between that of the placebo and that of the instillation of thio-TEPA in preventing recurrences of stage I bladder tumors. Pyridoxine was included as a treatment in this clinical trial because it is known to correct an innate metabolic abnormality in tryptophane metabolism which has been detected in as many as 50% of patients with stage I bladder tumors in other studies. Until now measurements of urinary metabolites of tryptophane have not been performed in this study, but since the results look so encouraging these studies will be undertaken early in 1975. Thus far recurrences have been observed in only 8 of 18 patients on oral pyridoxine compared to 16 out of 19 for placebo and 12 out of 19 for topical thio-TEPA. On the other hand the recurrence rates expressed in number of instances of recurrence per patient month of follow-up have been 0.065, 0.089, and 0.048, respectively for the three treatments. If the present trends continue, we may be able to show for the first time that a vitamin is useful in preventing a recurrence of cancer.

In addition to the clinical trials described above, the Section provides statistical support for ancillary studies performed on these same patients. I have already described above the analysis of some biochemical measurements done

in collaboration with the VACURG Reference Laboratory in Minneapolis. Another study involves measuring zinc concentrations in sera, hair, and needle biopsies and correlating the latter with the histological appearance of the biopsy. Studies have been done in collaboration with the Trace Elements Research Laboratory at the Washington Veterans Administration Hospital. In the last year we have been able by regression techniques using the data from the needle biopsy specimens to show that the concentration of zinc in cancer of the prostate is dramatically lower than that of normal prostatic epithelium. The technique employed in these studies is to divide the needle biopsy lengthwise and perform histological examination on one half and atomic absorption spectrophotometry on the other half. It now appears that this technique combined with the statistical analysis developed in our Section may be of general use in other problems where the localization of some substance in the components of a mixed tissue is important.

The Section has devoted a great deal of attention to collaboration with Dr. Donald Gleason in working out and testing a grading system for prostatic cancer. This has involved constructing and editing a data set containing information on over 3000 patients from the VACURG studies to whose tumors he has assigned a histological grade. Combining information on the histological grade and stage has resulted in prognostic categories which provide very reliable information about probability of death due to prostatic cancer. This approach has been so successful that these prognostic categories will be used in primary stratification of treatments in the next main VACURG clinical trial, Main Prostate IV (see below).

Two additional projects in the past year have been concerned with metastasis in patients with prostatic cancer. In the first of these, pre-treatment x-rays were reviewed for 103 patients from the first VA study who had metastasis at the time of diagnosis. The site and an estimate of the extent of metastasis was recorded for each patient. Analysis of these data, presented at the International Cancer Congress in Florence, has revealed that these more detailed observations provide highly useful information beyond the simple recording of bone metastasis as either present or absent. This work is being continued in an effort to develop a measure of response to treatment based on the distribution and appearance of metastasis in follow-up x-ray studies. This matter is of some importance since a truly measurable lesion is not usually present in patients with prostatic cancer. For this reason treatment comparisons are usually based on survival statistics. This approach, though it has many advantages, is time-consuming and comparisons are often clouded by later changes of treatment. Development of objective measures such as a combined index of extent and distribution of bone metastasis should prove useful in comparing chemotherapeutic agents such as those to be examined in the new studies discussed below. The second study of metastasis was based on a review of autopsy protocols from some 500 patients. We were able to show a definite correlation between histological appearance of tumor at the time of diagnosis and the probability of metastasis in all major sites. In addition it was observed that probability of metastasis in all major sites was decreased

by estrogen therapy compared to placebo, demonstrating that estrogen has definite effects on the tumor.

Significance to the Program of the Institute:

This Section serves the members of the Veterans Administration Cooperative Urological Research Group by assisting in preparing and revising protocols, forms, and records; giving statistical guidance in the design and execution of clinical trials; processing and analyzing data that is received daily on patients in the various studies; and assisting the members of the Group with reports on the progress of the various studies.

In return for these services, the Clinical and Diagnostic Trials Section has access to the largest complete set of data available anywhere on the course of prostatic cancer. Data on more than 4,300 patients with prostatic cancer has been accumulated with follow-up now approaching 15 years for some of the early patients. These data are proving extremely useful and interesting not only in the comparison of treatment effects, but also in defining the general behavior of prostatic cancer in relation to a host of personal, clinical, and laboratory characteristics.

A further advantage to the Institute is that the actual day-to-day experience of serving as a statistical support center for several large scale clinical trials provides an ideal environment for identifying important methodological questions of general applicability to the design, conduct, and analysis of clinical trials.

Proposed Course of the Project:

The cooperative studies undertaken jointly by the Veterans Administration and this Section are planned to be a self-generating sequential series to continue as long as fifteen years. Consultative services of this Section have expanded considerably over the past years as the data from Main Prostate I and II have ripened and as patients have accumulated in Main Prostate III.

On April 1, 1975, Main Prostate IV will be begun. In this study treatment assignment will be based on the combined clinical stage and histological grade. Patients in the lower categories will be randomized between placebo, 1.0 mg. DES daily and 1.0 mg. DES daily plus 300 mg. of aspirin twice a day. It is hoped that aspirin might possibly further reduce the cardiovascular complications in patients with carcinoma of the prostate since it is known that aspirin can reduce platelet aggregation and may thus be of value in preventing arterial thrombosis. Additionally it is known that in some experimental systems aspirin can retard the formation of metastasis in animal tumors. Patients in the high stage-grade categories will be randomly assigned to either 1.0 mg. DES daily, Flutamide, 250 mg. three times a day, or a combination of the two. Flutamide (SCHERING 13521) is a new anti-androgen which probably exerts its effect on prostatic cancer by a mechanism different from that of estrogen. It is thought that the anti-androgen blocks

the growth effects of testosterone and other androgens on prostatic epithelial cells possibly by inhibiting the formation of an essential dihydrotestosterone-protein-chromatin complex in the prostatic cell nucleus. Flutamide is a nonsteroidal drug which is apparently devoid of estrogenic, progestational, androgenic, or adrenal cortical activity. In addition, anti-androgens are not known to effect the cardiovascular system adversely, therefore, these agents are potentially ideal for the treatment of prostatic carcinoma. This drug has not yet been tested in a large-scale clinical trial. The second new protocol, to be designated the Chemotherapy Study, is designed to admit patients who are in relapse from previous hormone therapy or orchiectomy. Patients will be randomized between one of the following three categories: 1) intravenous diethylstilbestrol diphosphate (Stilphosterol) followed by oral stilphosterol, 2) oral Estracyt, and 3) L-phenylalanine mustard (L-PAM). The logic of this trial is to compare a pure estrogen (Stilphosterol), an estrogen bound to a nitrogen mustard (Estracyt) and a pure (non-estrogenic) nitrogen mustard derivative (L-PAM).

Although all members of the Section are involved to some extent in the activities described above, these projects have been the main responsibility of the Section Head. In addition members of the Section are involved in other projects concerned with investigating various aspects of general methodological interest, especially as appropriate to analyzing data from clinical and diagnostic trials. Dr. Larry Muenz, a Ph.D. in statistics, completed some studies on the distribution of the Mantel-Haenszel chi-square statistic when used pseudo-sequentially in comparing survival distributions. This work was submitted for publication. In addition, he was involved as a consultant and co-author in studies related to the treatment of peripheral malignant melanoma by hyperthermic perfusion and consulted on analysis of data related to asbestos as an environmental carcinogen. In september 1974 Dr. Muenz went to Lyon, France, where he will be working for two years at the International Agency for Research in Cancer as an exchange scientist from the National Cancer Institute. Dr. James A. Koziol, a Ph.D. statistician, joined the staff of the Section in November. Since that time he has continued work based on his Ph.D. dissertation concerned with multivariate rank statistics and has submitted two papers for publication on this subject. In addition, he has worked out the percentage points for the asymptotic distribution of one and two-sample Kolmogorov-Smirnov tests for truncated or censored data. This work has direct application in comparing survival distributions when not all patients have died or in testing the adequacy of theoretical models for survival compared to the observed data. He is currently extending his work on statistical tests for censored data by investigating the effect of random censoring on the Cramer-Von Mises statistic. This work should be extremely useful in competing risk problems. Dr. Mitchell Gail, an M.D. with considerable sophistication in statistical methodology, has continued his work in advising the immunodiagnosis committee on the statistical aspects of their contract proposals and has helped in designing and assessing the planned clinical trials of melanoma and coccidiomycosis. His most recent theoretical work has been concerned with setting a cut-off value for new diagnostic
significance levels used to demonstrate the effectiveness of such tests of

choosing the optimal cut-off point with respect to minimizing errors of misclassification. In addition to this and other theoretical work, Dr. Gail has analyzed several specific sets of data including data on immunodiagnostic tests for cancer and medical time series data on testosterone levels.

Members of the Section have been involved in other activities as site visitors and consultants to various programs outside the branch. The Section Head is a member of the Breast Cancer Task Force Treatment Subcommittee and Chairman of the Etiology and Prevention Subcommittee of the National Prostatic Working Cadre. In addition he has recently been appointed to the Interagency Task Force on Reserpine and Breast Cancer and to the Task Force for staging urogenital cancers of the American Joint Committee on Staging and End Results. Dr. Gail has acted as a statistical consultant to the NCI Committee on Immunotherapy and the NCI Committee on Diagnostic Radiology. He is a member and statistician for the NCI Committee on Immunodiagnosis.

During the last year we have continued our contract with the Mason Research Institute (formerly Wolf R&D Company) who assist us in analysis of the large amounts of data at our disposal by providing programming assistance. We are currently using a full-time systems analyst and two full-time programmers. This contract has been extended for another year. This relationship has been surprisingly convenient and productive.

Honors

David P. Byar, M.D.

Invited Address, American Statistical Association, St. Louis, August 26-29, 1974. "Selecting Optimum Treatment in Clinical Trials Using Covariate Information"

Invited Address, Department of Statistics, University of New York at Buffalo, September 23, 1974. "Clinical Trials of Treatment for Prostatic Cancer Including an Exponential Survival Model and Selecting Optimum Treatment Based on Covariate Information"

Invited Address, School of Public Health, University of Pittsburgh, December 3, 1974. "Clinical Trials of Treatment for Prostatic Cancer Including an Exponential Survival Model and Selecting Optimum Treatment Based on Covariate Information"

Panel Discussion, American Urological Association Meeting, Miami Beach, May 11-15, 1975.

Appointed to an Interagency Task Force on Reserpine and Breast Cancer, October 1974.

Appointed to the Task Force on Cancer of Urological Sites of the American Joint Committee for Cancer Staging and End Results Reporting, March 1975.

Mitchell Gail, M.D.

Invited Address, Department of Biostatistics, University of Washington, Seattle, October 3, 1974. "Theory and Application of Competing Risk Analysis to Survival Data"

Invited Address, American Statistical Association, Washington Chapter, Washington, D.C., November 6, 1974. "Theory and Application of Competing Risk Analysis to Survival Data"

Invited Address, National Center for Health Saatistics, HEW, Rockville, March 14, 1975. "Theory and Application of Competing Risk Analysis to Survival Data"

James A. Koziol, Ph.D.

Appointed to the Committee on Special Invited Papers of the Institute of Mathematical Statistics, October 1974.

Publications

Bayard, S., Greenberg, R. and Byar, D. P.: Comparison of treatments for prostatic cancer using an exponential-type life model relating survival to concomitant information. Cancer Chemotherapy Reports 58: 845-860, 1974.

Blackard, C. E., Byar, D. P., Seal, U. S. and Doe, R. P.: Correlation of pre-treatment serum nonprotein-bound cortisol and total 17-hydroxycorticosteroids values with survival in patients with prostatic cancer. New England Journal of Medicine 291: 751-755, 1974.

Byar, D. P.: Zinc in male sex accessory organs: distribution and hormonal response. In Structure and Function of the Male Sex Accessory Organs. (Brandes, D., Ed.) Academic Press, Inc., 1974, pages 161-171.

Byar, D. P.: Contributions of the Veterans Administration Cooperative Urological Research Group studies to our understanding of prostatic cancer and its treatment. In Urologic Pathology - The Prostate. (Tannenbaum, M., Ed.) Lea-Febiger (In press).

Gail, M.: Power computations for designing comparative Poisson trials. Biometrics 30: 231-237, 1974.

Gail, M.: Value systems for comparing two independent multinomial trials. Biometrika 61: 91-100, 1974.

Gail, M.: Measuring the benefit of reduced exposure to environmental carcinogens. Journal of Chronic Diseases (In press).

Gail, M.: A review and critique of some models used in competing risk analysis. Biometrics (In press).

Gail, M. and Ware, J.: Value systems for comparing three or more multinomial trials. Biometrika (In press).

Greenberg, R., Bayard, S. and Byar, D. P.: Selecting concomitant variables using the likelihood ratio stepdown procedure and a method of testing goodness of fit in an exponential survival model. Biometrics 30: 601-608, 1974.

Hovsepian, J. A., Byar, D. P. and the Veterans Administration Cooperative Urological Research Group: Correlation between radiologic quantitation of metastases and patient survival in carcinoma of the prostate. Urology (In press).

Mantel, N. and Byar, D. P.: Evaluation of response-time data involving transient states: an illustration using heart-transplant data. Journal of the American Statistical Association 69: 81-86, 1974.

Stehlin, J. S., Jr., Giovanella, B. C., deIpolyi, P. D., Muenz, L. R. and Anderson, R. F.: Results of hyperthermic perfusion for melanoma of the extremities. Surgery, Gynecology and Obstetrics 140: 339-348, 1975.

Serial No. ZO1-CP-04265-10-B

1. Biometry Branch, OADFS&S Division of Cancer Cause and Prevention
2. Mathematical Statistics and Applied Mathematics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Consulting in Statistics and Applied Mathematics

Previous Serial Number: Same

Principal Investigators: John J. Gart, Hugh M. Pettigrew, Robert J. Connor, Donald G. Thomas, M.W.J. Layard, Robert E. Tarone

Other Investigators: Jun-mo Nam, Alroy M. Smith

Cooperating Units: None

Man Years:

Total:	4.5
Professional:	4.0
Other:	0.5

Project Description

To collaborate with NCI scientists on the applications of mathematical statistics and applied mathematics to the interpretation and analysis of scientific experiments.

Objectives:

The principal objectives are (1) to collaborate with NCI scientists on mathematical problems related to cancer research, (2) to provide consulting assistance in statistics and applied mathematics to NCI investigators, and (3) to accelerate the use of quantitative methodology in various aspects of the NCI intramural program and extramural program.

Methods Employed:

The methodology of applied mathematics, mathematical statistics, and probability is applied to biomedical problems. Often variations of existing techniques are developed to suit the special requirements of a particular problem.

During this year the staff advised and collaborated with many investigators in the major divisions of research in the National Cancer Institute as well as some outside contractors and other investigators. The various projects are grouped below in terms of the Divisions and Areas of the projects.

Office of the Director, NCI

Dr. Tarone and Dr. Gart prepared a peer review of the statistical aspects of "The implications of cancer-causing substances in Mississippi River water" by Robert H. Harris of the Environmental Defense Fund. The review was prepared at the behest of the Deputy Director of NCI and made available to the public health authorities in Louisiana.

Division of Cancer Control and Rehabilitation

Dr. Connor is serving as the principal statistical consultant for the Breast Cancer Detection Demonstration Project, a joint ACS-NCI effort. The Project has 27 screening centers in 24 states and at each center 10,000 women between the ages of 35 and 75 are to be entered into the screening program. The program includes an initial screening and four annual recall screenings; at each screening three modalities are employed (i.e., physical examination, mammography, and thermography). Each participant is to be followed for five years after the last annual screening. The Project was designed primarily to demonstrate that breast cancer screening could be applied to the general population. Nevertheless, there are several research goals included in this program. The major research undertaking is a case-control study to investigate risk factors and to define the group of women who should be screened.

Dr. Connor has been actively involved in many facets of the Project. He has served on site visit teams evaluating the screening centers, participated in meetings of the Project's Protocol Review Committee, been an invited speaker at the semi-annual conferences of the center's directors and been assisting in the design of data forms as well as in the preparation of statistical reports. His main activity, however, is in connection with the case-control study. He has been responsible for the design of the study from the development of an interview questionnaire to defining the sampling procedures for the selection of controls.

Dr. Connor is expected to continue his full participation in this Project until the DCCR can assign a statistician to it. At that time his efforts would be mainly confined to the case-control study.

Division of Cancer Treatment

At the behest of Dr. Seymour Perry, Deputy Director, Division of Cancer Treatment, Dr. Gart did a statistical analysis of the animal experimentation involving the potential chemotherapeutic agent, Amygdalin, popularly known as Laetrile.

Division of Cancer Cause and Prevention (DCCP): Tobacco Working Group

Dr. Layard has assisted in the analysis of the large scale mouse skin-painting experiments conducted under NCI's Smoking and Health Program. He has provided consulting assistance to the Director of the Program, Dr. Gio B. Gori, and to the prime contractors, Enviro Control Inc. He made a presentation of the results of the second skin-painting experiment to the Tobacco Working Group at its September 1974 meeting.

Mr. Thomas had numerous consultations with Mr. Mike Rowley of Enviro Control. During the year, full responsibility for data processing on the Tobacco Condensate Study was transferred from this section to Enviro Control. Several of the computer programs developed by Mr. Thomas and Mrs. Smith are now being used by Enviro in this study.

Dr. Gart wrote the chapter "The Statistical Analysis of the First Mouse Skin Painting Study" for a monograph entitled Toward Less Hazardous Cigarettes Report No. 1, The First Set of Experimental Cigarettes.

Division of Cancer Cause and Prevention (DCCP): Director's Office

Dr. Gart performed a statistical analysis of the variation of the SV40 transformation test among control individuals and inhabitants of three Italian towns with high cancer rates. This work was done in conjunction with Dr. Robert DePue of the Director's Office, Division of Cancer Cause and Prevention.

Division of Cancer Cause and Prevention (DCCP): Carcinogenesis

Dr. Pettigrew has continued his collaboration with Dr. C. Wesley Dingman of the Chemistry Branch to develop stochastic models for DNA synthesis and repair. A manuscript based on this work has been submitted for publication.

Dr. Tarone collaborated with Dr. Harry Gelboin and Dr. Friedrich Wiebel of the Chemistry Branch in the analysis of a double blind study concerning the reproducibility of measurements of Aryl Hydrocarbon Hydroxylase induction levels in cultured human lymphocytes. Dr. Tarone evaluated measurements of AHH induction level with regard to use as a medical screening device and use in further experimentation.

Mr. Nam and Dr. Gart have been analyzing the results of a feeding study in rats and mice of Chlordane and Heptachlor in collaboration with Dr. Norbert Page. This Gulf South study was conducted under contract to the National Cancer Institute. These chemicals attracted such intense public interest that a preliminary report on analysis of carcinogenesis has been released. Following the complete pathology review, a final report will be prepared.

Mr. Nam and Dr. Gart continue to be involved in the statistical analysis of a feeding study in rats of seventeen environmental chemicals. They are collaborating with Dr. E.K. Weisberger and Dr. Norbert Page on this experiment, which was done at Litton-Bionetics.

Dr. Pettigrew has continued to collaborate with Dr. David G. Kaufman of the Carcinogenesis Area and H. Robert Baird of the Division of Computer Research and Technology in the development of an interactive computer system to retrieve and analyze data stored in the Carcinogenesis Bioassay Data System.

Dr. Gart participated in a "Workshop on Chemical Mutagenesis" held in June 1974 under the aegis of the Carcinogenesis area at NIH. In conjunction with Dr. Lionel Poirier of the Carcinogen Metabolism and Toxicology Branch, he set up some statistical methodology for analyzing in vitro and host mediated bioassay systems for mutagenesis.

Dr. Pettigrew has continued his collaboration with members of the Lung Cancer Branch on a variety of topics:

He is completing the analysis of data from an experiment carried out by Dr. David Kaufman, Lung Cancer Branch, and Dr. Peter Swann of the Middlesex Hospital Medical School to study the production of kidney tumors by DMN and MMS.

He is collaborating with Dr. Arthur Frank on the analysis of data from trachea culture experiments, studying various sources of variation, in order to design more precise experimental procedures for future experiments.

He is advising Dr. David Reese and Ms. Dianne Newton on sampling procedures for obtaining data from cell labelling experiments in organ culture.

Dr. Connor is working with Dr. Curtis C. Harris, Lung Cancer Branch, DCCP, on research on the metabolism of benzo(a)pyrene(BP) by cultured bronchial mucosa. Compounds known to inhibit the metabolism of BP in experimental animal cells are being examined to see if they alter the level of $[^3H]$BP found in DNA in the human bronchial mucosa.

Dr. Connor, in collaboration with Dr. Curtis C. Harris, Lung Cancer Branch, DCCP is analyzing data from a study of alpha$_1$-antitrypsin and lung cancer. Preliminary analyses are completed and final analysis will be started when more data are available. It is anticipated that this study will be completed this year.

Mr. Nam and Dr. Gart have completed a series of statistical analyses on three feeding animal studies to investigate a carcinogenic effect of saccharin in collaboration with Dr. E.K. Weisburger and Dr. Norbert Page. Rat and mouse studies at Bio-Research Institute and a rat study at Litton-Bionetics were involved.

Dr. Gart and Mr. Nam reviewed a draft on "Evaluation of the Carcinogenicity of Chloroform" prepared by Dr. Herman Kraybill of the Carcinogenesis Area.

Mr. Nam has consulted with Dr. Richard Yamamoto of the Experimental Pathology Branch in the analysis of data from his rat experiment which attempts to find the role of Vitamin B12 on certain carcinogens.

Dr. Connor has continued to advise Drs. Robert Bast, Edward Leonard, Monte Meltzer, Herbert Rapp and Berton Zbar of the Biology Branch on problems in the design and analysis of their experiments.

Dr. Gart reviewed the statistical methodology used in an experiment on the effect of caffeine on chemical carcinogenesis for Dr. Joseph DiPaolo, Head of the Cytogenetics and Cytology Section of the Biology Branch.

Dr. Connor continued to serve as the statistician for the University of Utah group studying the effect of chronic immunosuppression on physical and chemical carcinogens. This work is being done on a contract from the Biology and Immunology segment, Carcinogenesis Program, NCI. He is responsible for the statistical analysis of experimental data and assists in the design of experiments. This year he has made site visits to the University and reported on his analysis of their results. Currently he is collaborating on a paper on various features of UV carcinogenesis.

Dr. Connor consulted with Dr. Barbara Detrick-Hooks, Biology Branch, on the analysis of a study comparing techniques used to measure complement mediated cytotoxicity of nucleated cells. He did the analysis and assisted in the interpretation of the results. This work is reported in a paper by Dr. Barbara Detrick-Hooks, Dr. Tibor Borsos, and Dr. Herbert J. Rapp.

Division of Cancer Cause and Prevention (DCCP): Viral Oncology

Dr. Pettigrew completed his work with Dr. Kelman Perk and Dr. Michael Chirigos of the Virus and Disease Modification Section, on the analysis of data obtained from spleens of mice treated with LSTRA and various drugs. The results of a new method of quantitating the effect of chemotherapy in the LSTRA system were reported in JNCI.

Dr. Pettigrew also collaborated with Dr. Wilna Woods of the Virus and Disease Modification Section on the analysis of data from experiments to study the effect of Levamisole on immune stimulation in vitro.

Dr. Layard is assisting Dr. U. Heine and Dr. G. Weber of the Viral Studies Section with a study of length measurement of the genome of RNA tumor virus. The genome is made up of RNA molecules, and the distribution of measured lengths of these molecules is multi-modal, suggesting the possibility that the high-molecular-weight RNA of the virus may be composed of a number of single-stranded subunit molecules, each having a certain length distribution. Maximum likelihood methods are being used to decompose the observed distribution of lengths into five component normal distributions.

Division of Cancer Cause and Prevention (DCCP): Field Studies and Statistics

Dr. Connor and Dr. Roger Williams, Epidemiology Branch, NHLI, are collaborating on a case-control study on the relation of reserpine use and breast cancer. They are the principal investigators for the project and are responsible for its design, implementation, analysis and report. The study will be based on 300-400 breast cancer patients and 900-1200 controls all of whom are participants in the ACS-NCI Breast Cancer Detection Demonstration Program.

Dr. Tarone assisted Dr. John Goldsmith of the Office of the Associate Director (FS&S) in the interpretation of path analysis models and in the evaluation of the utility of such models in applications to environmental and occupational health hazards.

Division of Cancer Biology and Diagnosis

Dr. Pettigrew in conjunction with Dr. D. Jane Taylor of the Breast Cancer Program Coordinating Branch, DCBD, and Dr. Norbert Page, of the Carcinogen Bioassay and Program Resources Branch, DCCP, has continued to provide statistical consultation of the design and analysis of experiments conducted under contract by Dr. Albert Segeloff of the Alton Ochsner Medical Foundation, New Orleans, Louisiana to investigate the possible synergism between radiation and estrogen administration in the induction of mammary tumors in AxC rats. Further experiments, using the optimal radiation dose established from work already complete are under way to use the AxC model system to investigate further aspects of mammary carcinogenesis.

Dr. Robert Oldham, Laboratory of Immunodiagnosis, and Dr. Connor are working together to develop statistical procedures and methods to be used in designing and analyzing the Laboratory's experimental studies. This collaborative effort is in its initial stages and is expected to continue throughout the year.

For Dr. Elaine Esber of Children's Hospital National Medical Center and the Immunology Branch, NCI, Dr. Layard provided an appropriate theoretical model and statistical analysis of the relation between the proportion of pathological cells in the peripheral blood of children with acute lymphocytic leukemia and the proportions of cells with B and T cell lymphocytic surface receptors.

Dr. Robert J. Connor completed analysis of the data from a series of experiments on the effects of various immunosuppressive factors on the growth and development of tumors initiated by Murine Sarcoma Virus. The experiments were performed at Litton Bionetics under the direction of Dr. David Lavrin on a contract from the Division of Cancer Biology and Diagnosis on which Dr. Ronald Herberman, Chief Laboratory of Immunodiagnosis, is the project officer. A paper reporting the results of this work is in preparation.

In collaboration with Dr. Pietro Gullino and Mrs. Flora Grantham of the Laboratory of Pathophysiology, Dr. Pettigrew published a paper reporting the results of a study of mammary tumor induction in rats by IV injection of NMU.

Dr. Pettigrew advised Dr. Gullino and Dr. Michael Gimbrone on the analysis of data from an experiment implanting fragments of tissue in the eyes of rabbits to determine their ability to stimulate vascularization.

Dr. Pettigrew advised Dr. Jean Rotherham of the Laboratory of Physiology on the analysis of data from gel electrophoresis experiments.

Dr. Layard consulted with Dr. Mearl F. Stanton, Laboratory of Pathology, on the analysis of an experiment involving the induction of squamous carcinoma in the lungs of rats by means of implanted pellets containing cigarette smoke condensate. A paper describing this research was presented at a conference on

pulmonary carcinogenesis held in Seattle in June 1974, and has since been published in the proceedings of that conference.

Dr. Layard has continued his investigations, for Dr. Mearl F. Stanton, Laboratory of Pathology, of suitable methods of analysis of a study of the relation between fibrous glass particle size and mesothelioma induction in rats' lungs. Mrs. Alroy Smith has assisted in this work by writing a computer program to summarize sample distributions of particle size. A paper dealing with this research is in preparation. Dr. Layard has commenced the analysis of a similar series of experiments which employ crocidolite asbestos fibres as a carcinogen.

Dr. Layard collaborated with Dr. Mearl F. Stanton, Laboratory of Pathology, on the analysis of data from a study of epidermoid carcinoma development induced in the lungs of rats by implanted beeswax pellets containing 3-methylcholanthrene A paper has been published on this research.

Dr. Pettigrew has consulted with Ms. Hilda Wexler and Ms. Suzanne Kerney of the Surgery Branch on a variety of topics. He has begun to collaborate with Dr. Murray Brennan, now at Massachusetts General Hospital, on a clinical study of hyperalimentation which will begin when the latter joins the Surgery Branch later in the year.

For Dr. Robert H. Yonemoto, Department of Oncology Surgery, City of Hope Medical Center, who was a visitor at the Surgery Branch, Dr. Connor analyzed the results of experiments on a study of the immune response to cancer antigens. Dr. Yonemoto is preparing a paper reporting these results.

Dr. Tarone consulted with Dr. Bruce Wetzel of the Dermatology Branch and Dr. Katherine Sanford and Mr. Gary Jones of the Laboratory of Biochemistry concerning the estimation of the distribution of intermitotic times for normal and neoplastic cell lines. Conditional probabilities based on the estimated distributions were utilized in an attempt to obtain a representative sample of cells from each phase of the mitotic cycle for chemical analysis.

During the year Dr. Connor has advised Drs. Cecil Fox, Katherine Sanford and Robert Tucker of the Laboratory of Biochemistry, on the analysis of their experimental data.

Dr. Tarone has consulted with Dr. Wetzel and Dr. Elaine Alexander of the Dermatology Branch with regard to the analysis and interpretation of experiments concerning the differentiation of B and T lymphocytes on the basis of density of microvilli.

Dr. Gart consulted on questions of statistical methodology from time to time with Dr. Jay H. Robbins of the Dermatology Branch.

Dr. Tarone has been assisting Dr. Sanford and Ms. Sandra Handleman of the Laboratory of Biochemistry with regard to the design and analysis of experiments pertaining to methods of differentiating normal and neoplastic cell lines on the basis of morphological characteristics of cell colonies.

Dr. Tarone provided additional statistical assistance to the following NCI scientists: Dr. Cecil Fox and Dr. Robert Tucker of the Laboratory of Biochemistry, Dr. Raul Braylan and Ms. Sheilah Sanders of the Laboratory of Pathology, and Dr. Wetzel of the Dermatology Branch.

Dr. Robert J. Connor served on the site visit team that reviewed the performance of the Central Statistical Group for a Collaborative Study in Early Lung Cancer Detection screening at the University of Cincinnati.

Miscellaneous

Dr. Pettigrew has participated in meetings of the Clinical Oncology Computer Systems Working Group.

Dr. Pettigrew has provided statistical advice to various scientists doing contract work for NCI, including Dr. Mary Henry of the Illinois Institute of Technology Research Institute, Dr. John Thomas of Battelle Northwest, and Dr. Kende of Microbiological Associates.

Dr. Pettigrew collaborated with Dr. Samuel Wells, Department of Surgery, Duke University School of Medicine, on a manuscript reporting the results of work performed by Dr. Wells when he was with the Surgery Branch, NCI.

Dr. Gart consulted with Dr. John G. Bieri, chief of the Nutritional Biochemistry Section of the NIAMDD on a logit analysis of per cent hemolysis of rat blood cells.

Dr. Gart participated in discussion with staff of the NCTR on the statistical methodology to be used in the evaluating of carcinogenesis experiments done at their facility in Arkansas.

Mr. Thomas served as the section's expert on high speed computing often consulting with other members of the section and branch on the latest computer techniques applicable to their problems. This year, he further improved the section's library of computer routines which are often used in the consulting activities of other members of the section.

Mrs. Alroy M. Smith continues to play an important supportive role in the data analysis of this section particularly in writing and implementing statistical analyses on the high speed computers.

External Reviewing and Refereeing

Dr. Connor reviewed a paper on environmental factors and carcinogenesis for Dr. Ruth L. Kirschstein, Deputy Associate Commissioner for Science, FDA.

Dr. Gart prepared a critical review of EPA reports on the public health benefits and risks of oxidation catalysts in automobiles for Dr. Boris Osheroff of the Office of the Assistant Secretary for Health.

Dr. Pettigrew served as a referee for Cancer Research, Dr. Tarone refereed for Journal of Chronic Diseases, Dr. Gart and Dr. Tarone refereed for Blood.

Refereeing for the Journal of the National Cancer Institute were Mr. Nam, Dr. Connor, Dr. Layard, Dr. Tarone, and Dr. Gart.

Significance of the Problem to the Institute:

Members of this section are assuming an essential role in much research within the National Cancer Institute. Their activities include not only statistical analysis but also in planning valid experiments.

Proposed Course of the Projects:

Several of the projects mentioned in the Major Findings section will continue. In particular the collaboration with the various projects in the Carcinogenisis Area, the association with the Laboratory of Pathology, DCDB, the Cancer Control Breast Cancer Detection Demonstration Program, as well as other projects are expected to continue.

Honors and Awards

Dr. Layard was an invited participant at the Third World Conference on Smoking and Health held in New York City in June 1975.

Dr. Tarone and Dr. Pettigrew were invited participants in the Third Annual Carcinogenesis Conference in Orlando, Florida in February 1975.

Dr. Pettigrew and Dr. Layard were invited participants in the Symposium on Experimental Respiratory Carcinogenesis and Bioassay in Seattle, Washington in June 1974.

Dr. Gart was an invited participant at the workshop on "Statistical problems in developing procedures for estimating risks to man from results of animal experiments", an NIEHS sponsored workshop held in North Carolina in October 1974.

Publications

Gart, J.J.: Letter to Editor, Brit. J. Cancer 30, in press, 1975.

Gullino, P.M., Pettigrew, H.M. and Grantham, F.H.: N-Nitrosomethylurea as Mammary Gland Carcinogen in Rats. Journal of the National Cancer Institute 54: in press, 1975.

Harris, C.C., Connor, R.J., Jackson, F.E., and Lieberman, M.W.: Intranuclear distribution of DNA repair synthesis induced by chemical carcinogens or ultraviolet light in human diploid fibroblasts. Cancer Research 34: 3461-3468, 1974.

Hirano, T., Stanton, M.F., and Layard, M.W.J.: Measurement of epidermoid carcinoma development induced in the lungs of rats by 3-methylcholanthrene containing beeswax pellets. Journal of the National Cancer Institute 53: 1209-1219, 1974.

Perk, K., Chirigos, M.A., Fuhrman, F. and Pettigrew, H.: Some Aspects of Host Response to Levamisole After Chemotherapy in a Murine Leukemia. Journal of the National Cancer Institute 54: 253-256, 1975.

Stanton, M.F., Layard, M.W.J., and Miller, E.: Squamous carcinoma of the lung from cigarette smoke condensate in implanted beeswax pellets. In Experimental Lung Cancer: Carcinogenesis and Bioassays, (ed. Karbe and Park). Springer-Verlag, Berlin, 1974.

Wells, S.A., Jr., Sherwood, L.M., Burdick, J.F., Hattler, B.B., Christiansen, C., and Pettigrew, H.: The Allografted Parathyroid Gland: Evaluation of Function in the Immunosuppressed Host. Annals of Surgery 180: 805-813, 1974.

Serial No. Z01-CP-04267-10-B

1. Biometry Branch, OADFS&S Division of Cancer Cause and Prevention
2. Mathematical Statistics and Applied Mathematics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Research in Mathematical Statistics and Applied Mathematics

Previous Serial Number: Same

Principal Investigators: John J. Gart, Robert J. Connor, M.W.J. Layard, Hugh M. Pettigrew, Donald G. Thomas, Robert E. Tarone

Other Investigators: Jun-mo Nam, Alroy M. Smith

Cooperating Units: None

Man Years:

Total:	4.5
Professional:	4.0
Other:	0.5

Project Description

Objectives:

To conduct basic research in mathematical statistics, probability and applied mathematics; to develop new statistical methodology which is especially appropriate to bio-medical sciences.

Methods Employed:

The methods employed are the modern theories of mathematical statistics, probability, and applied mathematics. High speed electronic computers are often used to compute appropriate mathematical tables and test approximations by simulation techniques.

Major Findings:

The Research of the members of this section covers a wide spectrum of topics in mathematical statistics, probability, and applied mathematics. These are summarized below.

1. Estimation of Gene Frequencies in Generalized ABO Systems

Jun-mo Nam and John J. Gart are investigating the extension of Bernstein's methods to generalized ABO systems, such as the HL-A system. Among the results shown are (1) the "improved" Bernstein method is not fully efficient in the general case, although it is in the special case of the ABO system, (2) a single iteration of C.A.B. Smith's gene counting method will yield a set of estimators which is always better than any of Bernstein's estimators, and (3) these results can be used in the efficient design of studies of the possible connection between HL-A gene frequencies and disease.

2. Tests of Survival Data or Tumor Incidence Data

Robert E. Tarone has used simulated sampling methods to investigate the small sample properties of various proposed chi-square tests for equality of censored survival (incidence) distributions. A paper detailing the results of this study has been submitted for publication. The results are useful in determining the appropriateness of these tests in analyzing animal tests for potential carcinogens.

3. Estimation of a Linear Regression when both Variables are Subject to Error

Maxwell W. Layard has continued his investigation of the large sample properties of normal theory maximum likelihood estimates under the normality assumption. Simulated sampling methods are used to compare the small sample properties of these estimates with those of other estimation methods.

4. Models for Mammary Tumor Systems in Rodents

Hugh M. Pettigrew has continued his efforts to develop models for mammary tumor systems in rodents, and is considering extensions of existing statistical theory that may be necessary to evaluate data from such systems.

5. Stochastic Model for Breast Cancer Screening

Robert J. Connor is developing a stochastic model to evaluate breast cancer screening programs. The model will permit screening policies such as periodicity of screening, combination of modalities employed (i.e., physical examination, mammography, and thermography) and rules for biopsy recommendation to be investigated. This research has been prompted by the ACS-NCI Breast Cancer Detection Demonstration Project. It is anticipated that this work will result in specific recommendations for breast cancer screening programs.

6. Pseudo-Random Numbers for Continuous Distributions

Mr. Thomas is investigating new and more efficient ways of easily generating pseudo-random numbers from general continuous distributions. This work should prove useful in Monte Carlo applications such as studies of robustness.

7. Analysis of Ratios and Cross-Product Ratios of Poisson Variates

John J. Gart has devised tests for ratios and cross-product ratios of Poisson

variates, both in simple and stratified cases. The methods may be applied to ratios of rates of rare diseases in differing geographic areas. The cross-product ratio tests are useful in testing whether a geographic-sex interaction may be significant. A paper applying the methods to skin cancer incidence has been submitted for publication.

Alroy M. Smith has written a computer program implementing these methods. The program has been made available to other sections of the Biometry Branch.

8. Estimating Inbreeding Coefficients in Codominant Systems

Jun-mo Nam has been studying the estimation of inbreeding coefficients in co-dominant genetic systems. Among the results already found are (1) Li and Horvitz's method, the computationally most simple estimator, is almost fully efficient when the coefficient is very small (which is typical in human populations), and (2) a statistic for testing the inbreeding coefficient is developed.

9. Maximum Likelihood Estimation

Robert E. Tarone has studied some of the problems of uniqueness involved in the consideration of multiparameter maximum likelihood estimators. A paper has been submitted for publication.

10. Logistic Model and Optimum Choice of Dosages in Bioassay

Jun-mo Nam has completed his investigation on logistic model and optimum choice of dosages in bioassay. The logistic function is derived from certain logical assumptions as a quantal response model. Optimal dosages in estimating the parameters of the dose response curve are obtained. The results may be very useful in designing an experiment where an investigator has some notion about ED50 and the slope from a prior experiment.

11. Approximate Tests for Trends in Bioassay Data

John J. Gart is investigating approximate tests for non-zero slopes, equality of non-zero slopes, and intercepts in the analysis of proportion data in one or several strata. The methods will prove useful in analyzing certain carcinogenesis experiments particularly when the numbers may be too large to use exact methods.

12. Human Health and Environment

Jun-mo Nam reviewed the U.S. mortality experience and its implication as well as environmental health problem areas and identification problems. The problems on health standards and some issues on the solution of human environment are studied.

13. Multiple Comparison Techniques

Robert E. Tarone has derived simultaneous confidence ellipsoids for all subsets of a given set of estimable parameters in a general linear model. A paper

based on this research has been submitted for publication.

14. Estimation and Detection of Cyclic Trend

Jun-mo Nam has been investigating the proper statistical methods to estimate and identify a cyclic trend. These methods may prove very useful in detecting a seasonal effect on disease incidence, e.g., clinical onset of acute lymphatic leukemia. It was found a technique proposed by Edwards is fully efficient when the variation is small. The results are being prepared for publication.

15. Tests for Correlation Matrices and for the Structure of Covariance Matices

Maxwell W. Layard is investigating the effects of non-normality on normal theory tests concerning correlation matrices(e.g. equality of two correlation matrices), and tests about the structure of covariance matrices (e.g. equality of variances and covariances). Monte Carlo methods are used to study the small sample properties of alternative test procedures.

16. Models for DNA Synthesis

Hugh M. Pettigrew, in collaboration with Dr. C. Wesley Dingman, is applying the theory of stochastic processes to the development of models to explain phenomena associated with DNA synthesis and breakage.

17. Tables for Comparison of Proportions

Donald G. Thomas and John J. Gart completed work on a paper entitled "Tables of exact confidence limits for odds ratios, ratios and differences in proportions". This paper should be especially useful to researchers without ready access to high speed computers. The paper has been submitted for publication.

18. Reviewing and Refereeing

Robert J. Connor reviewed a research proposal on nonparametric inference for quantal response for the Committee on Basic Research, Advisory to the U.S. Army Research Office.

Maxwell W. Layard served as a reviewer of a grant proposal for the National Science Foundation.

John J. Gart served as a reviewer for Science Books: A Quarterly Review.

John J. Gart served as reviewer of a grant proposal in Applied Mathematics and Statistics for the National Science Foundation.

Maxwell W. Layard, Robert J. Connor, and John J. Gart served as referees for Biometrics. John J. Gart and Maxwell W. Layard served as referees for the Journal of the American Statistical Association. Maxwell W. Layard served as a referee for Technometrics and John J. Gart refereed for Communications in Statistics.

The interplay between mathematical theory, data analysis and experimental research is an important element in biomedical research. Many of the "Major Findings" reported above are new statistical techniques which have or may be directly applied to data collected by the medical researchers at NCI or other workers on problems in cancer research. Others are mathematical models which may elucidate the biological phenomena involved in cancer research and which may also aid in the planning of subsequent experiments or epidemiologic studies. The opportunity for initiating fundamental research on mathematics and mathematical statistics is essential for enabling members of the section to achieve professional recognition among their peers in their own scientific disciplines. More importantly, the possibility of doing such unconstrained research is a prerequisite for the consulting work of the section to be carried out at the highest professional level.

Proposed Course of the Project:

Many of the projects described in the major findings will be continuing, e.g. breast cancer screening models in human, mammary tumor models in rodents, research on censored survival tests and correlation matrix tests, estimation of frequencies and inbreeding coefficients in genetic models, and generation of pseudo-random numbers. Other research, not referred to above, shall also be initiated; it will include the development of new statistical methods and mathematical models in various biomedical problems that come to our attention during the year.

Honors and Awards

John J. Gart was appointed Associate Editor of Biometrics.

John J. Gart was appointed to the program committee for the International Biometric Conference to be held in Boston in 1976.

John J. Gart continues to serve on the Nominating Committee of the American Association for the Advancement of Science (Section U: Statistics).

John J. Gart continued to serve as the American Statistical Association representative to the Biometric Society, Eastern North American Region.

John J. Gart completed his term as a member of the Regional Committee of the Biometric Society.

Jun-mo Nam gave an invited paper entitled "Logistic Model and Optimum Choice of Dosages in Bioassay" at Mathematics Session, Allied Science and Engineering Symposium, Seoul, Korea in July 1974.

Jun-mo Nam gave an invited talk on "Human Health and the Environment" to Allied Science and Engineering Symposium, Seoul, Korea in July 1974.

John J. Gart presented an invited paper entitled "The analysis of ratios and cross-product ratios of Poisson variates with applications to incidence

rates", at the annual meeting of the Biometric Society in St. Louis in August 1974.

John J. Gart presented an invited paper entitled "The Poisson distributi The theory and application of some conditional tests" at the International Cc ference on Characterizations of Statistical Distributions and Their Applicati to Theoretical Statistics and Applied Fields" in Calgary in August 1974.

John J. Gart was an invited lecturer at the Department of Biostatistics of the University of North Carolina in March 1975.

Publications

Arvesen, J.N. and Layard, M.W.J.: Asymptotically robust tests in unbalanced variance component models. Annals of Statistics, 4: in press, 1975.

Gart, J.J.: Exact moments of the variance test for left-truncated Poisson distributions. Sankhya, Series B, 36: in press, 1974.

Gart, J.J.: The Poisson distribution: The theory and application of some conditional tests. Proc. Conf. Stat. Distns. Sci. Work (G.P. Patil, S. Kotz, and J.K. Ord, editors), Vol. 2, in press, 1975.

Layard, M.W.J.: A Monte Carlo comparison of tests for equality of covariance matrices. Biometrika 61: 461-465, 1974.

Layard, M.W J.: Book review: "The Generalized Jackknife Statistic" by H.L. Gray and W.R. Schucany. Journal of the American Statistical Association, 70: in press, 1975.

Nam, J.: Logistic model and optimum choice of dosages in bioassay. Proc. of the Integrated Symposium on Sci. and Engr., Seoul: in press, 1975.

Nam, J.: Human Health and Environment. Proc. of the Integrated Symposium or Sci. and Engr., Seoul: in press, 1975.

Thomas, Donald G.: Exact and asymptotic methods for the combination of 2X2 tables. Computers and Biomedical Research, 9: in press, 1975.

Serial No. Z01-CP-04268-08-B

1. Biometry Branch, OADFS&S
   Division of Cancer Cause and Prevention
2. Special Cancer Survey Section
3. Bethesda, Maryland

NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Third National Cancer Survey

Previous Serial Number: Same

Principal Investigators: John L. Young, Jr. and Sidney J. Cutler

Other Investigators: Roger Connelly, Susan Devesa, John Horm, James Larson, Constance Percy, Joseph Scotto, Valarie Van Holten, Roger Williams

Cooperating Units: University of Alabama School of Medicine
Georgia Regional Medical Program
State of Colorado Department of Health
University of Texas Southwestern Medical School
Michigan Cancer Foundation
University of Iowa
Mayo Clinic
University of Minnesota, Department of Epidemiology, School of Public Health
University of Pittsburgh, School of Public Health
Puerto Rico Commonwealth Health Department
State of California, Department of Public Health
Iowa State University
Mason Research Institute
Epidemiologic Pathology Unit, NCI
Electronic Data Processing Staff, BB, NCI

Man Years:

Total:	6
Professional:	6
Other:	12

Project Description

Objectives:

To prepare a complete register of all new cancer cases diagnosed in the resident population of seven standard metropolitan areas, two states, and the Commonwealth of Puerto Rico during the period 1969-71, plus all other cancer cases seen by doctors or hospitals in those areas; to obtain demographic data and a description of the tumor for each patient; and to study certain

economic, social, and other aspects of the disease in a sample of patients.

Methods Employed:

In each survey area, NCI contracted with local organizations to hire field staff and gather data. Data were requested from doctors and obtained from hospital records and death certificates. With approval of their doctors, a sample of cancer patients were interviewed. Local staffs did the preliminary editing and coding of data. Institute staff in Bethesda, Maryland, completed the editing and coding of data and converted it to magnetic tape for use in preparing detailed reports through electronic computers.

Basic information was collected on every active cancer case, except certain skin cancers, during the three-year period. This included race, age, place and date of birth, sex, and marital status. Medical information included the primary site and histologic type of tumor, date and method of diagnosis. Information available from any existing cancer register or continuing survey in a survey area was utilized to avoid duplication of effort. All reports were treated as strictly confidential.

Death certificates mentioning cancer were obtained from the appropriate vital statistics offices. Local survey staff or hospital personnel abstracted information about cancer patients from hospital records.

Trained interviewers, after receiving written permission from attending doctors, obtained from a selected sample of patients information on the amount of disability incurred, time lost from productive activity, cost of treatment, and sources of payment. Questions were also asked regarding religion, number of children, socioeconomic status, and personal habits such as smoking and drinking. Hospital records on this selected population were reviewed for more detailed information on cost of hospitalization, methods of meeting this cost, treatment for the cancer, and the extent of the disease at time of diagnosis.

Workshops were conducted periodically to resolve problems which the supervisors had encountered. The format of round table discussions on specific questions and informal discussions among the supervisors proved very effective in carrying out the instructional and communicative objectives of these workshops.

Extensive use was made of computer capabilities in the processing of the survey documents. Each document was processed separately, with the computer tagging and combining all the records for an individual patient. The computer conducted a large number of edit checks--consistency among documents on the same patient, as well as among items on the same document. The sample selection was also a computer operation with the printing of the appropriate labels included in the operation. The data processing time was reduced to a minimum. Within five weeks after a record was received at NCI, the data were converted on to computer tapes, edit runs were completed, and printouts of error reports and updates of survey control reports were mailed back to the field offices. Data were accessed on a weekly cycle and collated into the master file. Each item was reviewed to insure that it was within set limits and that the coded information was a valid code. Other edits included

determination of consistency or validity between items on the same abstract form. The computer also checked for consistency in data among the various forms on the same patient. There was a feedback to the survey areas of any invalid, inconsistent, or questionable data. The survey field offices researched the problems and corrected the data stored in the computer tapes. The data were reviewed periodically to insure that corrective action had been taken by the survey field office.

As the survey approached the completion of the data-gathering operation, a variety of reports were being produced to assist in assurance of complete reporting and accuracy of the information. These included (1) listing of cases that required followback, such as death certificate abstracts, to insure accuracy of the data; (2) listing of cases that required supplemental data on medical care and hospital costs to insure complete reporting of these cases; and (3) special listing of cases with erroneous or questionable data to insure that the cases were researched and corrections made.

Because of limited staff available, some of the quality control procedures were modified. The reinterview program was eliminated because of the problems in obtaining the reinterviews, and the lack of feedback either to the survey field office staff or the NCI. The reabstract program was also modified by eliminating the reabstracting of a random sample.

The last batch of basic records was received in the central office from the fields in November, 1973. In January, 1974 a tape was created which contained data extracted into a single record for each patient from all basic documents ever received on a given patient, except that, if a patient had two or more cancers active during 1969-71, an extract record was created for each case. A similar extraction for sample data is discussed below.

A special skin cancer survey was started September 1, 1971, which collected data on cases diagnosed through February 29, 1972. Only four of the survey areas--Iowa, Dallas, Minneapolis, and San Francisco--were participating in this special study. Special efforts were made to contact all physicians who would see or treat skin cancer patients to obtain their cooperation. Pathology laboratory files supplied data on cases that were histologically diagnosed. In addition, abstractors completed separate forms for skin cancer patients found during their normal visits to participating hospitals. Data collection has been completed and magnetic tapes have been created to use in data analysis.

The last batch of sample documents was submitted to the central office in March, 1974. Patient interviews were submitted in July, 1974. These data have been extensively edited and extracts containing patient interview data, hospitalization financial data and hospitalization treatment data have been or are being created.

From March thru August, 1974 the remaining field office core staff assisted central staff in assigning census tract to the address at which each patient lived at the time of diagnosis. Whenever possible, Census Bureau DIME files were used, but approximately 40 percent of the cases had to be tracted by hand. This work has been completed and all field offices were closed as of August 31, 1974. In field offices not continuing in the SEER Program (see NCI-4254)

local copies have been put into storage to be accessioned only through the contract's project director. In those field offices continuing in the SEER Program - Detroit, Iowa, San Francisco-Oakland and Puerto-Rico - the local records have become part of the SEER case files.

Major Findings:

An advanced report based on the 1969-1971 incidence data for all areas combined has received wide distribution. Reports specific for each field office have been distributed locally by each field office principal investogator. The three-year report for all areas combined differed from the 1969 Preliminary Report in two major respects: (1) data for all of Colorado rather than the Denver SMSA were included, and (2) in situ carcinomas were excluded from every site.

An NCI Monograph is in press at the Government Printing Office (NCI Monograph 41, March 1975). This monograph contains detailed incidence data by race, sex, and age of the patient and primary site of the cancer for each geographic area participating in the survey - except for Puerto Rico - and for all areas combined. In addition the monograph contains two complex tables showing a distribution of the cancers classified by histologic type within primary site and also primary site within histologic type. These innovative tables will be useful to researchers investigating specific sites and or types of cancer, e.g., angiosarcoma of the liver or mesonephromas of the cervix and vagina, etc. The monograph also presents tables showing numbers of cases of in situ carcinoma diagnosed in each of the survey areas by sex and race. However, these cases have not been included in the calculation of incidence rates presented in the monograph. Highlights from the monograph are presented below:

1) Number of cases - The seven metropolitan areas and two States included in the survey had a combined population of 21 million in 1970. During the 3-year period 1969-71, this population generated 181,027 new cases of cancer, exclusive of in situ carcinomas and nonmelanoma cancers of the skin. An additional 58,586 cancers were diagnosed before 1969 among residents who received medical care during the period 1969-71. During the same period, 31,452 new cancers were diagnosed among persons residing outside of the survey areas.

The number of cases diagnosed among residents of each of the survey areas varied from 6,243 in Birmingham to 34,609 in Detroit. This variation was generally proportional to the population residing in each of the areas.

2) Diagnostic accuracy - Of the 181,027 cancers diagnosed during the survey period, 90.1% were known to have had a microscopically confirmed diagnosis.

The percentage of microscopically confirmed diagnoses varied widely according to primary site. The sites for which at least 98% of the cancers were microscopically confirmed were: corpus uteri (99.3%), penis (98.9%), testis (98.6), melanoma of the skin (98.4%), thyroid (98.1%), soft tissue (98.1%), and Hodgkin's disease (98.0%). In contrast, less than 70% of cancers of the following sites were microscopically confirmed: primary site not specified (69.6%), pancreas (69.5%), and "large intestine, segment not specified" (60.5%). It is interesting to compare these low percentages with the overall percentage

of 73.5 in 1947. The marked upward shift in the percentage of microscopically confirmed cancer diagnoses provides a rough measure of the improved quality of medical practice in the United States.

The percentage of microscopically confirmed cancer diagnoses varied moderately among the nine survey areas. It was at its lowest (86.1) in Pittsburgh and its highest (93.1) in Minneapolis-St. Paul and San Francisco-Oakland.

3) Variation by race and sex according to primary site - One of every four cancers occurs in the digestive system. This is true for males and females, whites and blacks. The relative frequency of cancers of the genital organs is roughly equal in the two sexes: 17.7% in males and 20.6% in females. In contrast, cancer of the breast accounts for 27.2% of malignant neoplasms in women and for only 0.2% in men. This sex differential is largely compensated for by that for cancers of the respiratory system, which accounts for 24.1% of malignant neoplasms in men and for 5.9% in women.

Examination of the incidence of cancer in specific organs discloses some striking differences among the four race-sex groups, for which data are given. For example:

Lung - The risk of developing lung cancer is highest among black men, an incidence of 89.9 compared with rates of 70.7 for white men and rates of 14.4 for both white and black women.

Prostate - Incidence of prostatic cancer is much higher in black than in white men (rates of 94.9 and 57.7, respectively).

Esophagus - Incidence of esophageal cancer is much higher in black men than in the three other race-sex groups (a rate of 16.7 in black men compared to rates of 4.7 in white men, 3.7 in black women, and 1.3 in white women).

Uterus - Invasive cancer of the cervix uteri occurs more than twice as frequently in black than in white women (rates of 33.6 and 14.9, respectively). In contrast, cancer of the corpus uteri occurs at a rate of 21.0 in white females compared to a rate of 12.1 in black females. The reversal between the races in the occurrence of cancer in the two anatomic segments of the uterus cannot be attributed to inaccurate classification. The proportion of cases with insufficient details regarding the location of uterine cancers was small in both groups. The rate for cancers classified as "uterus, not otherwise specified" was only 2.3 for whites and 2.7 for blacks.

Breast - Cancers of the breast occur more frequently in white than in black women (rates of 75.0 and 57.6, respectively).

Bladder - White males have the highest risk of developing bladder cancer (a rate of 23.5 in white males compared to rates of 12.2 in black males, 6.2 in white females, and 4.3 in black females.

3) Variation by age - The age pattern of cancer incidence differs in the two sexes. Before age 25, the incidence of cancer (all forms combined) is low among both males and females. From age 25 to age 55, the incidence of cancer increases more rapidly among women than among men. Among women, the rate increases from 54 per 100,000 at ages 25-29 to 508 per 100,000 at ages 50-54. Among men, it increases from 35 per 100,000 at ages 25-29 to 415 per 100,000

at ages 50-54. From age 55 on, the increase is much more rapid in men so that, at ages 80-84, the rate is 2,832 per 100,000 in men compared to 1,504 per 100,000 in women.

The data also showed that among the survey areas cancer of the lung, prostate digestive tract, breast, and uterus demonstrated geographic differences.

Findings from the special skin cancer survey indicated that the estimated incidence of the site is grossly understated. Even in the survey area with the lowest incidence rate tabulations indicated more than twice the number of cases than expected. Because of this large increase of cases over expecte the data were reviewed with a number of consultants including a committee appointed by the National Academy of Science at the request of the Institute. However, the report of this committee has not been completed to date.

Individual papers on cancer incidence in children, in U.S. blacks, variation by geographic area, and papers on the incidence of retinoblastoma, melanoma, non-melanoma skin cancers, polycythemia veria, cervical cancer and cancers of the large bowel have been published and/or presented at national and international meetings. A paper presenting an overview of available data has been published and reprints widely distributed in an effort to make the medical public aware of the availability of survey data.

An Advisory Committee for Utilization of Data from the Third National Cancer Survey met in August, 1974 to assist the staff in handling requests for data by outside research groups. As a result a public use tape containing non-confidential data on the 181,027 incidenct resident cases of cancer has been created and is available for purchase by medical researchers at a cost of $100.

Significance of the Program to the Institute:

Registration of all cancer cases in a defined population is the only way to acquire reliable data on cancer incidence rates. The rates themselves are indicators of the significance of cancers in the various sites or of different types. This data bank will provide a valuable source for epidemiolo investigations by identifying population groups that have a high or low incidence of cancers of specific sites. Some of the factors that will be studied include residence, occupation, place of birth, use of contraceptive devices, and the use of tobacco and alcohol.

Other products of the survey that will be important to the mission of the National Cancer Institute will be studies on the delivery of medical care to a large and representative sample of cancer patients, treatment provided the cancer patient, extent of disease at time of diagnosis, direct and indirect costs of cancer, and the extent and duration of disability from cancer.

The survey has produced unique data which can be used by other investigators as a starting point for more intensive investigations. One area of special interest is rare tumors. Because of the low numbers of these tumors, epidemiologists feel that investigations will yield significant results and possible etiologic leads.

The data contained in the monograph are a valuable source of information not available anywhere else in the country. Daily, a large number of queries are received on cancer sites and histology to which we are now able to respond with data. The information has also been useful in program planning and in preparing reports for NCI, NIH and HEW use. The survey data have also indicated there were errors in estimates of incidence by site used by various cancer agencies. The data will enable us also to provide a more sensitive method for determining estimates of the incidence of cancer in the future. Further, the availability of incidence data has enabled Institute staff to assist other program areas, particularly the Cancer Control Program, in site reviews and contract awards.

Proposed Course of the Project:

The data collection and processing operations have been completed. All field offices were closed as of August 31, 1974 except for those continuing in the SEER Program - Detroit, Iowa and San Francisco-Oakland. Tapes containing basic incidence information about the site, type, date and method of diagnosis of the cancer have been created and were used in the preparation of the monograph and papers mentioned above. A public use tape has also been created. Extract tapes containing sample data concerning hospital treatment and financial information and the epidemiological data from the patient interview have also been created and are currently being analyzed. A number of papers utilizing these data are being planned. Financial data from the patient interview remains to be edited and extracted.

A monograph containing detailed data on the incidence of non-melanoma skin cancer is being prepared. This monograph will compliment Monograph 41 from which the non-melanoma skin cancers were excluded.

Analyses of cancer incidence by marital status, urban/rural residence and county of residence is expected to be completed during the next 12 months. An analysis of incidence by socioeconomic status using census tract data is progressing. However, many difficulties have been encountered in merging TNCS files, second count census population files and fourth count (sample data) census tract demographic files so that socioeconomic indicators such as median family income, median education and percent below poverty level can be assigned to cancer patients and aggregated across all field offices. It is anticipated that difficulties arising from surpressed data on the census files can be overcome and that the planned socioeconomic analyses can also be completed in the next twelve months.

Analyses of cancer incidence among Spanish surnamed persons is expected to progress and these data will be compared with incidence data from Puerto Rico. Incidence data from Saskatchewan Canada and from several SEER areas - Connecticut Utah and New Mexico - will be compared to TNCS incidence data.

Restructuring of data files from the Second National Cancer Survey (1947-48) is being completed at which time trends in age-specific incidence rates for various sites among the four major race-sex groups will be studied. This analysis will be of use in making more accurate projections of cancer rates in the future.

Due to limitations of available computer programming staff as well as analytical staff, all analyses discussed above may not be completed within the next fiscal year.

Honors and Awards:

Lectures:

Sidney J. Cutler "Demographic Patterns of Cancer Incidence in the United States" Workshop of Persons at High Risk of Cancer, Key Biscayne, Florida, December 6, 1974.

"Payments to Hospitals for the Care of Cancer Patients" Annual Science Writers Conference, American Cancer Society, San Diego, California, March 21, 1975.

"Current USA Cancer Statistics" Cancer Centers Conference, Durham, North Carolina, April 10, 1975.

"Who Are We Missing and Why" National Conference on Advances in Cancer Management - Detection and Diagnosis, Denver, Colorado, May 1, 1975.

Joseph Scotto "Incidence of Malignant Melanoma of the Skin in the United States, 1969-71" XIth International Cancer Congress, Florence, Italy, October 24, 1974.

John L. Young, Jr. "Benefit Cost Analyses in the Health Field" Department of Health Administration, Duke University, July 31, 1974.

"Report on the Third National Cancer Survey" USA-USSR Joint Working Group for Cancer Epidemiology, Bethesda, Maryland, February 10, 1975.

"Available Data from the Third National Cancer Survey" Interagency Collaborative Group on Environmental Carcinogenesis, Bethesda, Maryland, March 4, 1975.

"Cancer in US Blacks" Conference on Nutrition in the Causation of Cancer, Key Biscayne, Florida, May 22, 1975.

Committee Membership:

Roger R. Connelly - Working Cadre of the National Program - Pancreatic Cancer.
Joseph Scotto - International Lung Cancer Group, Founding Member

Publications:

Cramer, DW and Cutler, SJ: Incidence and Histopathology of Malignancies of the Female Genital Organs in the United States. American Journal of Obstetrics and Gynecology, 118:443-460, February, 1974.

Cramer, DW, Cutler, SJ, and Christine,B: Trends in the Incidnece of Endometrial Cancer in the United States. Gynecologic Oncology, 2:130-143, March, 1974.

Publications: (continued)

Berg, JW, Ketelaar, RJ, Rose, EF, and Bernon, RG: Hepatomas and Oral Contraceptives. The Lancet, p. 349, August 10, 1974.

Scotto, J, Kopf, AW, and Urbach, F: Non-Melanoma Skin Cancer Among Caucasians in Four Areas of the United States. Cancer, 34:1333-1338, October, 1974.

Mason, EE, Nance, M, Radcliffe, CE, Scotto, J, and Berg, JW: Skin Cancer in Iowa. Journal of the Iowa Medical Society, 469-479, November, 1974.

Cutler, SJ, Scotto, J, Devesa, SS, and Connelly, RR: Third National Cancer Survey - An Overview of Available Information. Journal of the National Cancer Institute, 53:1565-1575, December, 1974.

Cramer, DW: The Role of Cervical Cytology in the Declining Morbidity and Mortality of Cervical Cancer. Cancer, 34:2018-2027, December, 1974.

Young, JL Jr and Miller, RW: Incidence of Malignant Tumors in US Children. The Journal of Pediatrics, 86:254-258, February, 1975.

Frenkel, EP, Clark, B, and Percy, C: Cancer Incidence Rates in the Dallas-Forth Worth Metropolitan Area. Texas Medicine, 71:71-78, March, 1975.

Biometry Branch: National Cancer Institute Monograph 41: Third National Cancer Survey: Incidence Data. Bethesda, Maryland, National Institutes of Health, US Department of Health, Education, and Welfare Publication No. (NIH) 75-787, March, 1975.

Cutler, SJ: Colon and Rectum -- Incidence and Mortality in the United States" in Cancer Epidemiology and Prevention (Shottenfeld, D., ed.) p. 375-385, Charles C. Thomas, Springfield, Illinois, 1975.

Devesa, SS: The Incidence of Retinoblastoma: Data from the Third National Cancer Survey. Journal of Ophthalmology. In press.

Devesa, SS, Young, JL Jr, and Williams, RR: The Incidence of Polycythemia Vera Among the Elderly. Journal of the American Medical Association (JAMA). In press.

Cutler, SJ and Young, JL Jr: Demographic Patterns of Cancer Incidence in the United States. In Persons at High Risk of Cancer: An Approach to Cancer Etiology and Control, (Fraumeni, J, Editor). In press.

Serial No. Z01-CP-04269-04-B

1. Biometry Branch, OADFS&S, DCCP
2. Automatic Data Processing Management Section (ADPMS)
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biomedical Computer Systems - Consultation and Services

Previous Serial Number: Same

Principal Investigator: Theodore Weiss

Other Investigators: Mary M. Cusano, Altamease A. Gales, Terence D.C. Kuch, Norman Oslik, J. Michael Stump, Valerie D. Van Holten, James E. Larson

Cooperating Units: None

Man Years:

Total:	15
Professional:	7.5
Others:	7.5

Project Description

The Automatic Data Processing Management Section provides consultation and assistance to the scientific and administrative staff of the National Cancer Institute, and as necessary, to other governmental agencies, private institutions, and individual investigators who collaborate with the National Cancer Institute in its mission. Consultation and assistance are given on individual, as well as on large-scale cooperative studies involving epidemiologic, laboratory and clinical investigations, cancer surveillance programs, cancer control projects, information and reporting systems for cancer centers, program planning and appraisal activities, and administrative management operations.

Objective:

To facilitate the research activities and other complex operations of the National Cancer Program through the application of computer science, information technology and operations research; by minimizing the organizational, operational, mechanical and logistical constraints arising from scientific investigations and related activities which involve data and information collection, purification, storage, computation, retrieval, analysis and dissemination.

Methods Employed:

Consultation, assistance and collaboration encompass four major activities:

1. Planning, Design, Organization, Administration and Evaluation: Consultation and participation in the planning, design, organization and administration of projects having data management and statistical computing requirements; establishing patient registries and statistical, data management and coordinating centers for large-scale cooperative studies; preparing study reports on the technical feasibility and budgetary implications of various organizational, operational and methodological approaches to programs and projects utilizing computing equipment, and data management and statistical computing systems and libraries; conducting individual site reviews and participating on technical selection and review committees for grants and contracts; assisting investigators and contractors identify sources of, and obtain, statistical and computer-related resources and services; developing training programs and conducting seminars and workshops on various aspects of biomedical computing;

2. Development, Implementation and Operation of Data Management and Statistical Computing Systems and Libraries: Providing consultation and technical assistance through forms design, systems analysis, systems design, and the preparation and distribution of procedure manuals; providing computer programming services using digital computers, scientific computers with analog-digital converters, and related electronic instrumentation; providing generalized software systems and libraries for investigators' own use and to expedite and simplify the use of computers; conducting workshops and providing individual instruction in the use of computer systems, computer programs and equipment; coordinating and monitoring a) the conversion of raw data into forms appropriate for computer processing and b) the day-to-day operation of data processing systems and related activities;

3. Operation of Remote Terminal Facility: Operating and maintaining a Remote Terminal Facility with such equipment as the IBM 2922 Batch Entry/ High Speed Printer Terminal, and IBM 2741 Typewriter Terminals;

4. Research and Development in Computing Methodology: Conducting research and development work in collaboration with computer scientists and specialists at universities and clinical centers throughout the country to develop new and improved methodologies in the application of computers to medicine.

Major Findings:

ADP Management Section

This has been another noteworthy year for the Section in terms of accomplishments within the Branch and in collaboration with investigators in other Divisions and Institutions.

Although the Third National Cancer Survey continued to require a sizable portion of the Section's resources this year, the SEER Program has become our primary focus. We anticipate that our activities and staff commitments to the SEER Program will increase over the next several years as the Program gets underway. The Section also provided consultation and assistance on a variety of continuing and special studies conducted by investigators in the Biometry Branch and the Epidemiology Branch.

Our consulting activities in the intramural and collaborative programs increased markedly as a result of the SEER Program and in response to requests from Branch and Lab Chiefs in the Division of Cancer Biology and Diagnosis and in the Division of Cancer Control and Rehabilitation. Many of the requests from other NCI Divisions had to be declined, however, because of the shortage of professional staff in the Section. Regardless of whether resources are placed in the Divisions themselves, or in the ADPMS, there is a critical need for additional staff at both the professional level and the mid-level to provide biomedical computer systems consultation and services in the Institute.

Most of this additional support can be obtained through contract services, but a core of NCI staff must administer these contracts. We are now exploring the use of "expert" and "consultant" positions and the Intergovernmental Personnel Act as possible mechanisms for alleviating some of these staff shortages. We have recommended that the other Divisions within the Institute utilize these sources too, and we have been asked to assist in the recruitment of their computer specialists. Two mid-level computer specialists were recruited for our Section this year to provide continuing services for the Third National Cancer Survey as it moves into the data analysis phase, and to develop the computer programs required by the SEER Program. A biolab technician was transferred from DCBD to the ADPMS for training in the computer field in order to provide additional computer services to that Division.

The nature of our consulting work has also expanded appreciably. In addition to systems analysis we are now applying techniques in management analysis and operations research. In collaboration with registry staff we are conducting comprehensive reviews of the organization and operation of several of the registries making the transistion from the End Results Program and the Third National Cancer Survey to the SEER Program. This is being done in order to assist the registries through a complex technical effort, and at the same time to modernize and streamline their operations. We are also consulting on the establishment of statistical, data management and coordinating centers for several large-scale cooperative studies and demonstration projects being conducted by the Division of Cancer Biology and Diagnosis and the Division of Cancer Control and Rehabilitation.

All of the professional staff have served on technical review committees for various grants and contracts. In spite of the travel freeze, and indicative of the extent of our consulting activities this year, members of the Section made almost forty trips to other institutions collaborating with the NCI.

The Generalized Reporting Program (GRP) supported by the Section continued to receive considerable use within the Biometry Branch and in other institutions

participating in our research programs. ADPMS staff used the software package to produce reports for several projects within the Branch, and the system was modified to run at the Detroit SEER Program Registry, Wayne State University computer facility, and at the University City Science Center computer facility for the DCBD/DCCR Breast Cancer Detection Demonstration Project.

The ADPMS has obtained a software system developed and maintained by the Office of Systems and Standards, Bureau of Labor Statistics to select, restructure, cross-tabulate, and display research data. The system, Table Producing Language (TPL), was designed to solve some of the problems inherent in the collection of large volumes of data from which cross-tabulations must be produced in a wide variety of formats. TPL is a very high level problem-oriented language designed for use by investigators without extensive experience with computers. Mr. Stump and Mr. Farnsworth Hester are evaluating the system's performance, comparing its features, operation and resource requirements, and costs with other tablemaking systems such as the Generalized Report Program, IRS, and SPSS. At the conclusion of this evaluation, a demonstration of the TPL system will be conducted for FS&S staff.

A Remote Terminal Facility has continued to operate under the direction of the ADPMS. The Facility (Remote 17) contains an IBM 2922 with card reader and high speed printer, several IBM 2741 communication terminals, and a keypunch/card interpreter. A production desk-type service is operated by the ADPMS. In addition, a central Landow-DCRT pickup and delivery service operates from the Facility. The amount of output received on the printer was increased significantly during the year by the installation of a faster (7200 BAUD) telephone line. Remote 17 is funded jointly by the National Heart and Lung Institute and the National Cancer Institute.

The Field Studies and Statistics Area

The ADP Management Section provided consultation and assistance to nine Biometry Branch projects during the year:

The Surveillance, Epidemiology and End Results Reporting Program

The Section's consultation and service activities in support of the SEER Program have increased considerably this year. (For a description of the project see SEER Project Report, Biometry Branch, NCI.)

Most of the registries in the SEER Program were originally participants in the Regional Medical Program, the End Results Program, or the Third National Cancer Survey. These registries have required considerable retooling to prepare for the SEER Program. SEER requirements to report incidence and expanded extent of disease and therapy, to use new classification and coding schemes, and to convert existing files and records to SEER codes and formats are some aspects of the Program that are indicative of the complexity and effort involved. The new participants are also involved in a considerable technical effort to develop the systems and procedures that are required to operate as a population-based registry.

Many of the registries require new methodology for virtually all aspects of their operations. This may include new organizational, operational and administrative structures and procedures and possibly new computer systems and equipment.

The ADP Management Section has provided technical consultation and assistance to many of the registries, to the NCI Coordinating Committee, and to NCI project officers on the reorganization, administration, and data management aspects of converting the registries for SEER participation.

This involvement has resulted in the development of a prototype data management and statistical computing system for large population-based tumor registries. The system developed by the San Francisco Bay Area Resource for Cancer Epidemiology and the ADPMS, for the California Tumor Registry (CTR), has significant implications for other SEER participants. It incorporates much of the methodology developed for the Third National Cancer Survey such as the use of the computer to resolve most of the anatomical site and histologic type conflicts encountered in the operation of a Registry, and other computerized techniques pioneered by the CTR such as automated patient record-linkage. The prototype system uses remote terminals with text-editing software, graphical plotters, microfiche document storage, and sophisticated statistical software packages. Although this technology is commonly used at many research institutions, including NIH, it is not yet in general use in SEER registries.

The adoption of this methodology and equipment by the tumor registries in the SEER Program is an important step forward from the card- or even tape-oriented batch processing computer systems presently in use. Many functions traditionally performed manually such as document control, death certificate matching and patient record-linkage and consolidation are accomplished on the computer with improved efficiency and accuracy. The registries' reporting, research, and training activities can be greatly enhanced by the improved response time and expanded capabilities of an up-to-date computing environment.

The ADPMS worked closely with other Biometry Branch staff members on the development of 1975 submission requirements and related codes and formats, and on the design and implementation of a computer-based system to edit, tabulate, and facilitate the analysis of data from this first submission.

A new contractor was selected this year to provide systems analysis and computer programming support for the SEER Program and the analysis of the Third National Cancer Survey data. Mr. Larson prepared the RFP for this contract, chaired the selection committee, conducted an orientation session with Ms. VanHolten for the contractor's staff, administered the contract, prepared task statements, and reviewed work performance.

Mr. Theodore Weiss has continued to serve as a member of the SEER Coordinating Committee which has the responsibility for the overall organization and coordination of all aspects of the starting phase of the project. He was also appointed Executive Secretary of the SEER Management Analysis Subcommittee,

which has met several times during the year to consider and recommend methods for improving the operational aspects of the registries and the SEER Program. This year Mr. Weiss made site visits to the registries in California, Connecticut, and Utah. In California he conducted a preliminary study and evaluation of the Registry's organization, data management and statistical computing systems, and computer hardware and programming resources. This study recommended a complete reorganization of the Registry's operations and resulted in the detailing of Mr. James Larson to the California Tumor Registry for four months to direct and conduct a detailed requirements analysis and general systems design effort, and to prepare a Feasibility Study Report, required by the State government. Mr. Weiss, Mr. Stump and Ms. VanHolten collaborated with Mr. Larson on the design of a new data management and statistical computing system for the Registry and on the preparation of the Feasibility Study Report.

The report contained specifications for the new computer system and recommended that the Registry change computer centers to gain access to a more powerful computer with remote terminal and text-edit capabilities. The report also concluded that the Registry should contract for biomedical computer specialists to replace the business oriented programmers provided by the State government's central resource. The report also projected the impact of these changes on staffing and budget requirements. Mr. Larson and Mr. Weiss provided extensive support to the Registry in the preparation of a contract workscope, an RFP for data processing services, a request to NCI for supplemental funds to implement the proposed changes, and other material required for approval of the project by the State's Department of Finance. Subsequently, Mr. Larson and Mr. Weiss worked closely for seven months with the CTR and the California State Departments of Health and Finance to secure approval to proceed with the reorganization. Messrs. Larson and Weiss are presently providing overall technical direction in the development and implementation of the computer system.

Mr. Weiss visited the Utah Tumor Registry to review the status of the major revisions being made to their data management and statistical computing system and operations which he had recommended during a previous visit. These changes included a significant expansion of the manual and computerized data edits, the improvement of methods for case finding, quality control, and case registration. Mr. Weiss also arranged for Mr. Larry Derrick, the computer specialist at the Registry, to work with Mr. Larson at the California Registry for approximately one week in order to determine whether any of the techniques being used in the California system could be applied to the new system being developed at the Utah Tumor Registry.

In the Connecticut Tumor Registry Mr. Weiss also conducted a comprehensive review and evaluation of the organization and operations. He recommended that a computer specialist be added to the Connecticut Cancer Epidemiology Unit at Yale University to conduct a detailed study of the Registry, develop a new system, prepare a report on the feasibility of utilizing a

new system and if approved to direct and participate in the implementation of a new data management and statistical computing system.

Mr. Weiss and Mr. Larson are presently collaborating with the Connecticut Tumor Registry and the Yale Connecticut Cancer Epidemiology Unit on the development of a new data management and statistical computing system which will incorporate many of the features of the California prototype system.

Mr. Michael Stump consulted with the New Orleans Tumor Registry on the adaptation of computerized inter-record edits and record consolidation techniques. He also designed and programmed a computer system to convert the Registry's existing case records to SEER codes and formats. Mr. Terence Kuch visited the New Orleans Registry to explain SEER requirements and to advise them on automated record handling and storage methodologies and on the use of microfiche for document storage.

Other Registry consultation included the installation of the ERG Survival System at the Iowa Tumor Registry by Mr. Stump and a review of the Seattle Registry's data processing systems and computer center by Mr. Larson.

The ADPMS organized and co-sponsored with the Field Liaison Section a two-day data processing conference for SEER participants which covered all apsects of data processing related to the organization and operation of population-based tumor registries.

The conference included presentations by each registry of their present organization and data management and statistical computing systems. Changes planned for the January 1975 submission were discussed in detail. Other topics of discussion included forms design, data entry techniques, use of an intelligent terminal, computer record-linkage, microfilming of source documents, data editing, consistency-checking, record consolidation, data file structure, error reporting and correction, patient follow-up by exception, routine and special reports, the California prototype system, the use of remote terminals, computer graphics, computer type-setting for publications, and security and confidentiality.

The conference served as an effective forum for the exchange of techniques between the Program's participants. The consensus of opinion following the workshop was that additional workshops will be required to explore all of the topics presented in much greater detail.

Ms. Mary Cusano developed a computer system to process the SEER January 1975 submission tapes. This system was used to copy, edit, correct, and produce listings and tables for data evaluation and analysis. The system uses many of the complex data edit routines developed for the Third National Cancer Survey including site and histology hierarchy and combination editing. In addition to designing and programming the computer system, Ms. Cusano coordinated the submission of the tapes, and the process used to correct errors on them. She is presently developing the specifications for another system to be used for the more complex January 1976 SEER data submission.

Ms. Valerie Van Holten consulted with other ADPMS staff members on the more complex aspects of registry computer systems design, specifically on the details of computerized techniques for site, histology, and extent of disease editing and record consolidation which she developed for the Third National Cancer Survey. She also participated in the evaluation of the January 1975 SEER data by computing rates for multiple primary neoplasms and interpreting variations from expected rates. In addition, she assisted the California Tumor Registry in the consolidation of its 1972-75 incidence cases with cases collected by the Third National Cancer Survey for the period 1969-71.

Third National Cancer Survey

The Third National Cancer Survey was the primary focus of the Section's consulting activities from the early planning phase of the study in the fall of 1967 through the completion of the data gathering and data purification phase in the summer of 1974.

The Survey's methodology was largely incorporated into a complex computer system which was designed, programmed, and operated for four years by this Section and its data processing contractors. The overall plans for the Survey's data processing system and design of the data collection forms were developed by Mr. Weiss in 1967. He also participated in the organization and implementation of the Survey's field offices and developed much of the methodology used for abstracting, coding, and error correction. He also directed the detailed design, programming, and early operation of the Survey's computer system. Mr. Terence Kuch designed the data keying and optical scanning portion of the system which captured 250 million characters of data over a six-year period, with an error rate of less than 1%. Mr. James Larson directed the daily operation and ongoing development of the data processing system for three and one-half years, and he planned and directed a major portion of the data management work required to close the Survey and prepare the data base for analysis and publication.

Over one million abstract documents for 350,000 cases of cancer were handled by the Survey's data processing system during the four-year data gathering phase. Separate computer systems were developed by the ADMPS to convert the data base from the format used for data gathering to one more appropriate for analysis and to calculate and format hundreds of statistical tables for publication using the Government Printing Office's computer-driven typesetting equipment.

This year the Section is participating in the data analysis and publication aspects of the Survey. This participation is at a lower level than previous years and of a different nature. While consultation during the past five years involved primarily operational planning, systems analysis, data management, and contract coordination and review, it currently involves principally statistical computing support.

Computer programming and systems analysis support is especially significant to this project because of the complication inherent in the handling of a data base comprised of over one million records. Existing general purpose data management and statistical software packages frequently used on other studies do not efficiently process files of this size. In addition, many functions performed manually in smaller studies must be accomplished on the computer in a study of this size. These functions include, for example, the geocoding of census tracts, the determination of the socio and economic status of cases by linking with U.S. Census files, and the identification of urban and rural residents by the computer analysis of patient address at diagnosis.

Specific consultation activities on the Survey this year included:

1. Incidence rates were computed by various demographic variables, such as marital status, urban-rural residence, socioeconomic group, Spanish surname, and by detailed place of residence at diagnosis (county and census tract). This involved the modification of existing computational and reporting programs and of the Survey's geographic base file of populations. The socioeconomic status of cases was determined by computerized linking of census tract data with U.S. Census Bureau population characteristics file to produce reports of variations in incidence by such items as median family income and education level. Incidence rates for Spanish surnames were determined by computer matching cases with the U.S. Census Bureau's file of Spanish surnames and by modifying existing rate computation and reporting programs to accommodate this variable. A special computer program was developed to determine the urban-rural resident status of cases in Iowa by analyzing the patient's address and comparing it with a file of urban and rural places in the State. It was also necessary to modify the Survey's geographic base file to include urban and rural population counts. Incidence rates by marital status and detail place of birth were produced using existing computational and reporting programs.

2. Incidence rates were computed for Puerto Rico and Saskatchewan, Canada which were not included in the original analysis. This required the development of two new data editing systems to process the records from these areas since their cases were not abstracted and coded with standard survey protocol. All data from Puerto Rico were abstracted from death certificates, and the Saskatchewan cases used different forms, codes, and methodology. These cases were edited, corrected, recoded, resubmitted, and processed by modified computational and reporting programs.

3. An analysis of trends in incidence rates between the 1947 and 1969-71 National Cancer Surveys was completed using computer programs which compared the age-adjusted incidence rates from both Surveys and identified statistically significant variations by site, race, sex, age, and other descriptive and medical characteristics.

4. The tabulation of hospital cost and medical treatment data for publication was accomplished using a system developed by the ADPMS. This system correlated cost and treatment data from up to 25 data abstract forms for a single case with diagnostic and descriptive data on the Survey's statistical file for a 10% sample of cases and produced approximately fifty reports in camera-ready form for publication.

Japanese Migrant Studies

The ADPMS continued to provide systems analysis and computer programming support to the series of Japanese Migrant Studies. Mr. Michael Stump served as the principal consultant on this project and Ms. Altamease Gales provided the computer programming support. Additional lung data were edited and used to update the Lung Survey database. Pathology data were added to selected records in the file and Gastric Ulcer data were edited and formatted using modified versions of the Japanese Migrant Study edit and update programs. Several customized programs were written to recode and format the resulting Gastric Ulcer file, and a generalized program was written to use investigator-supplied parameters to match each case with its most comparable control from among the study participants. The complete study file was then formatted so that data retrieval could be accomplished using the Generalized Report Program.

Study of Blood Alcohol Levels

Mr. Stump and Ms. Gales provided consultation and programming services to Dr. Bernard Fox in a study of social characteristics of individuals with varying levels of alcohol concentrations in their blood. The study cohort consisted of a population selected from Howard County, Indiana and from Grand Rapids, Michigan. Mr. Stump and Ms. Gales designed and produced numerous tables plotting social attributes against varying blood alcohol concentrations. The study was a collaborative effort between Dr. Fox, ADPMS staff, and the Bureau of Alcohol Prevention. Results from the analysis of the data were presented by Dr. Fox to the 6th International Conference of Alcohol and Drugs held in Toronto, Canada.

International Classification of Disease for Oncology (ICDO)

The United States, in cooperation with the World Health Organization, is preparing a cancer supplement for the 9th Revision of the International Classification of Disease code. The International Classification of Disease for Oncology (ICDO) will consist of topography codes based on the malignant neoplasms of ICDO and an expanded morphology code based on the American Cancer Society MOTNAC. To assist the WHO in reviewing and revising the code structure, Mr. Stump and Ms. Percy designed and wrote computer programs to produce matrixes showing the malignant and benign counterparts for each existing term. These were used at a recent meeting of the World Health Organization in Geneva, Switzerland where an international committee convened

to draft the ICDO. Mr. Stump will continue to collaborate with Ms. Percy through the adoption and publication of the new code and he will direct the conversion of files required by the adoption of the new code.

International Death Certificate Study

Mr. Michael Stump collaborated with Ms. Constance Percy and staff members of the Office of Health Statistics on a study to determine variations in the cause of death coding between the United States and six other participating countries. The study cohort for the International Death Certificate Study consists of approximately 1200 mortality cases selected from the Third National Cancer Survey file. Death certificates for these selected cases were pulled from the manual files, duplicated and forwarded to health organizations in each participating country responsible for coding the cause of death. Upon receipt of the returned data, Mr. Stump designed reports and wrote computer programs to compare each country's certificates against the Third National Cancer Survey standard and against the coding supplied by each other participating country. Preliminary results revealed that statistically significant variations in coding do exist not only from country to country, but within the United States itself. The results of the ongoing analysis are being used to develop rules that should lead to more comparable neoplastic mortality coding in both the United States and in the international community. Mr. Stump will continue to participate in this study.

The Japan-Hawaii Cancer Study

The Japan-Hawaii Cancer Study was begun in 1971 to conduct interpopulation and case-control studies involving Japanese and Caucasians in Hawaii and Japanese in Japan. (See Japan-Hawaii Cancer Study Project Report, Biometry Branch, NCI for a description of the research project.) Mr. Norman Oslik has been involved with the project since its inception as the principal consultant.

The data management system designed and implemented at the Kuakini Hospital and at the University of Hawaii Computer Center under Mr. Oslik's direction has continued to operate during the past year in a routine and satisfactory manner. The National Information Processing System (NIPS) generalized data management package has been in continuous use on this project for over three years, providing regular monthly editing and updating of data, and has produced a wide variety of routine and special reports to facilitate the operation of the project, and the analysis of the data. Recent enhancements to the NIPS system have significantly lowered costs for both routine production and data analysis. The computer system has the capability of adding to and retrieving data from approximately 40 study forms, including personal history questionnaires, results of laboratory tests, pathology reports and hospital surveillance. The system will continue to evolve as refinements are made to the data collection forms based on an ongoing analysis of the value of the data collected to date. In addition, changes can be expected as data collection shifts to the relatives (siblings, spouses, etc.) of the original cohort.

Subjects have been entering the clinic for the Third Examination cycle since October, 1971. Approximately 8,000 people have been seen to date. All data ascertained through a subject's routine clinic exam, with the exception of certain lab results, are entered into the computer file within six weeks of their collection. After each monthly update of the file, computer-generated letters containing various test results are produced and sent to the subjects and their doctors.

Over the past year Mr. Oslik has functioned primarily in the role of a consultant to the JHCS systems analyst in Honolulu, providing assistance on problems in the areas of systems design, forms design, clerical procedures and the recruitment of data processing personnel.

In addition to the above, Mr. Oslik devoted a good deal of work toward the design of a family linkage subsystem. Family linkage represents a method of coding cases so that specific family groups (e.g., children, siblings, spouses) can be retrieved at one time for familial disease studies. Given the large number of cases in the study, it is felt that a computerized coding scheme is the only feasible approach to this aspect of the study.

Mr. Oslik will continue to have an active consulting role in this project.

The Ultraviolet Radiation Study

The Ultraviolet Radiation Study (UVR) is a cooperative project between Temple University, the Department of Transportation, and the National Cancer Institute. Mr. James Larson directed the design, programming, and operation of a computer system to capture, edit, store, and produce tables for the analysis of ultraviolet radiation exposure data gathered at one-half hour intervals at ten stations located around the world. This system uses the key-to-tape process to convert exposure data contained on continuous paper rolls to magnetic tape. The system analyzes the patterns of the incoming data to determine whether they follow logical exposure patterns and expected values. Significant variations are noted for review and possible correction. The system also supplies data for missing and incomplete days by calculating the average exposure at each one-half hour interval of the last recorded day before the gap and the first recorded day after the gap. Ms. Carol Parsons and Ms. Ruth Wolfson are providing operational support for this ongoing study. This involves the manual editing of exposure tapes and summary cards received monthly from ten stations, coordinating the data conversion and error correction process, and running the system edit, update, and reporting programs monthly.

Six computer programs have been written for the statistical analysis of the data. These programs perform such functions as computing average daily exposure by station, hour of the day, and season of the year and total cumulative exposure between specified hours of the day and calendar periods.

Epidemiology Branch Support

Since 1968 the ADPMS has been providing consultation, computer programming, and operational support to the Epizoology Section's Veterinary Medical Data Program. Originally, this system of computer programs, written and used at Michigan State University, was modified and transferred to DCRT by Mr. Oslik. The computer system is used to collect, store, retrieve, and analyze pathological and clinical procedure data on patients from fourteen university-affiliated veterinary clinics in the United States and Canada. Over the years the basic system has been further modified by members of the staff to accommodate an expanded data collection form, to incorporate more extensive data edits, to produce additional reports, expedite processing, and reduce operational costs.

During the past year Mr. Oslik has provided occasional consultation to Dr. William Priester and Dr. Howard Hayes. This has involved the detection and correction of minor flaws in the ongoing computer system.

The Viral Oncology Area

Mr. Weiss has continued to consult intermittently with Dr. Paul Levine, Immunology Epidemiology Segment, and Mr. Roger Connelly. Mr. Weiss participated in several review committee meetings and discussions with one of the Segment's research contractors in order to develop a source of systems analysis, computer programming, and operational support services for the Segment. Consultation is expected to continue on an intermittent basis.

OD, DCCP Administrative Management Branch

The ADP Management Section has continued to operate and enhance the Division's ADP Management Reporting System. This automated system generates a series of reports each month from a magnetic tape furnished by the Division of Computer Research and Technology (DCRT). They are distributed approximately two weeks after the end of each month. The reports provide information on monthly and year-to-date costs incurred at DCRT for systems analysis and computer programming services, data conversion (e.g., keypunching, key-to-tape), computer usage, terminal rental and telephone line charges for terminal usage. Information is summarized by : Unit; Section; Branch, Laboratory or Office; Area, and for the Division as a whole. At the present time three reports are being prepared: (1) Expenditures by Account and Project; (2) Terminal Utilization Report; and (3) Expenditures by Programmer/User. Annual allotments for each DCRT account are established in July for that same fiscal year, following the distribution of the final reports for the preceding fiscal year. These annual allotments are revised in January following the distribution of the December reports.

Mr. Weiss continued to serve as the Division's ADP Coordinator. He has the responsibility for preparing a sizable report each year for the Department, on the Division's expenditures for computer-related services at DCRT, for

computer-related equipment in use within the Division, for computer systems under development or in use within the Division, and for all Division contracts for data processing services.

Division of Cancer Biology and Diagnosis

The ADPMS consulted with investigators in the Division of Cancer Biology and Diagnosis on the following projects:

Radiation Branch

Dr. Ralph Johnson, Chief, Radiation Branch requested assistance to develop and implement a computer system to improve the methods used in managing his Branch's research data. The Radiation Branch is primarily involved in the development and evaluation of new cancer treatment techniques using irradiation alone and in combination with other modalities.

Mr. Michael Stump designed a computer system to collect, edit, store and retrieve protocol data. When implemented, the computer system will provide for on-line data collection through the use of a touch-sensitive or light-sensitive cathode ray tube (CRT) device. The visual data displays will "step" a physician, nurse or technician through the history-taking and recording of treatment and other information by a series of catalogued decision-tables. This technique of data gathering will provide multiple choice lists of responses to questions and will provide alternative lines of questioning depending upon specific responses to previous questions. The collected data will be formatted and stored on WYLBUR disk files. Automation of these data will facilitate the monitoring of patients' responses to treatment, and the exchanging of data on the collaborative studies carried out by the Branch and other program areas.

Mr. Stump has prepared an RFP for the Radiation Branch to obtain commercial data processing services to implement and maintain this system. He will participate in the selection of a contractor, in contractor orientation, in consultation on the system's specifications during implementation, and in evaluating the results of the final system's testing. Thereafter, Mr. Stump will provide consultation in this area on an intermittent basis. Implementation of this system has been extensively delayed due to several administrative problems encountered in obtaining contract services.

Surgery Branch

The Surgery Branch has the responsibility for providing surgical consultation and operative assistance to the NIH's intramural clinical programs, while conducting its own program of laboratory and clinical research. Ongoing investigations can be categorized in four principal areas of interest: cancer of the soft tissue pelvic structures, head and neck area, soft tissue and bone sarcomas, and melanomas. Dr. Steve Rosenberg is Chief of the Branch.

Much of the ADPMS's consultation with the Surgery Branch was provided by Mr. George Jenkins under the direction of Mr. Michael Stump. Mr. Jenkins was previously a laboratory technician in the Surgery Branch who had acquired some data processing experience through his own interest and initiative. Dr. Rosenberg reassigned him to the ADPMS during this fiscal year so that Mr. Jenkins could learn computer programming and systems analysis through an accelerated training program and by working in a data processing environment. This will increase his value to the Surgery Branch as he continues to participate in the development of solutions to their data management problems.

At Dr. Rosenberg's request, the ADPMS consulted with the Surgery Branch on three projects: (1) the design of a simplified protocol data management system; (2) the development of a computer system for research projects requiring the hyperalimentation of patients; and (3) the design of a system to facilitate the study of bacteria flora. The ADPMS also assisted in the recruitment of a computer aide for the Surgery Branch to operate their protocol system.

An earlier version of the Branch's protocol data collection system consisted of a lengthy questionaire prepared by attending physicians on all patients treated on the surgery service. Dr. Rosenberg requested that this system be streamlined to gather a smaller quantity of data that would be used primarily as an index to the patient's medical record. Mr. Stump and Mr. Jenkins provided consultation to the Branch in defining a core of data to be recorded on all patients and to be stored in computer files. Mr. Stump prepared the computer program specifications for the revised system. Ms. Valerie Van Holten provided copies of computerized routines to perform various complex edits of the data. Mr. Jenkins worked closely with the Branch's medical staff to design the data collection form for the system. This entire package was delivered to the Division of Computer Research and Technology for programming and implementation. The ADPMS plans no further involvement in this project.

Mr. Jenkins is also the primary consultant to Dr. Demetrius Bagely, Clinical Associate, Surgery Branch in the development of a system to store and edit data collected as part of a study of bacteria flora. The purpose of this project is to conduct a retrospective study to determine whether the prophylactic administering of antibiotics affects the normal flora of bacteria in selected sites for patients who have undergone radical cancer surgery in the Clinical Center. Mr. Jenkins designed a data collection form suitable for the recording of the research data and Mr. Stump advised Dr. Bagely on various data storage techniques and on the most appropriate data retrieval packages available. Mr. Jenkins also trained Dr. Bagely in the use of the retrieval system for the data analysis.

Mr. Stump has provided consultation to Dr. Rosenberg and Dr. Murray Brennen on a project to establish a Hyperalimentation service in the Clinical Center. This service will be involved in the intravenous feeding of patients having

undergone radical surgery. Results of laboratory tests will be collected daily and used to monitor patient progress as well as to evaluate the nutritional solutions administered. Thusfar, Mr. Stump has served to identify the potential sources of automated laboratory data in the Clinical Center and to advise on the design of the data collection system to be used to store the research data. He recommended that the Surgery Branch obtain data processing services from DCRT or a commercial contractor to further develop and implement the data collection system. Mr. Stump intends to continue to provide consultation only until other data processing services are obtained.

Immunology Branch

Mr. Stump consulted with Dr. William Terry, Chief, Immunology Branch, and members of his staff, on the further development of a computer system that is presently being used to support an on-going study of malignant melanomas. The Immunology Branch is primarily involved with basic immunobiology laboratory investigations, with particular emphasis given to studies of the structure and function of cell membranes, cellular immunity, genetics of the immune response system, and transplantation.

Mr. Stump had previously been the consultant on the team responsible for developing the Malignant Melanoma Protocol, comprised of medical staff from the Immunology Branch and a statistical consultant from the Biometry Branch. Mr. Stump has now designed a computer retrieval and display component for the system that will use a cathode ray tube device (CRT) to allow investigators to retrieve laboratory test results specified by the investigator, and display these values for a specified patient, for a selected subset of patients, or for the entire patient population under study. Implementation of this new component of the system will, for the first time, allow Immunology Branch investigators to automatically display the time-course of an individual's experience while under treatment, for comparison purposes with that of another study participant, or with the entire study population.

Mr. Stump has prepared an RFP for the Immunology Branch to obtain commercial data processing services to implement this new component of the system. His involvement in this project will continue through the selection and orientation of a contractor, consultation on the system's specifications during implementation, and evaluating the results of the final systems testing.

Metabolism Branch

Dr. Thomas Waldmann, Chief, Metabolism Branch,requested assistance to identify areas in which his research activities could benefit from the use of computer systems. The clinical research program of the Metabolism Branch is directed toward two major goals, (1) to define host factors that result in a high incidence of neoplasia, and (2) to determine the physiological and biochemical effects that a tumor produces on the metabolism of the host.

The Metabolism Branch does not currently utilize computerized data storage and retrieval systems to aid in its research program. Mr. Stump conducted a

general review of the Branch's data processing requirements, and prepared a set of recommendations and plans for the development and implementation of a computerized data management system. The report included a recommendation that a commercial data processing contractor be used to implement and maintain the computer system. Implementation of such a system, and the creation of a computerized database would allow the Branch's investigators to use the existing DCRT statistical and mathematical computer program libraries, and their computerized graphic equipment for data analysis. Further consultation with the Metabolism Branch is anticipated if Mr. Stump's recommendations are implemented.

Division-wide Consultation

As a result of the ADPMS consultation within the Division of Cancer Biology and Diagnosis, and some rather extensive discussions with their Branch and Lab Chiefs and their staffs, Mr. Weiss and Mr. Stump recommended and coordinated the establishment of a Clinical Oncology Computer Systems Working Group. This group is now providing a forum where investigators interested in the application of computers to cancer-related research are exploring the commonality of their biomedical computing problems, and taking advantage of the fairly extensive collaboration and interdigitating of clinical services and activities, are now developing common solutions to these problems. It is also facilitating the exchange of ideas and computing technology.

Division of Cancer Control and Rehabilitation

Diagnosis Branch, DCBD

The Demonstration Project for the Early Detection of Breast Cancer is a joint National Cancer Institute-American Cancer Society effort. NCI's activities were initiated by the Division of Cancer Biology and Diagnosis and have been funded by the Division of Cancer Control and Rehabilitation. The project began in fiscal 1972 and is expected to cover an eleven-year period.

The ADP Management Section continues to have a very active role in many areas of the project, including participation in overall project planning, coordination and direction, administration of a data management contract, forms design, OMB clearance, printing and distribution, computer systems planning, development and review of quality controls methods, the design of statistical reports, contract review of the screening clinics, conference and workshop planning, and assisting the various Advisory Committees and Working Groups associated with the project. Mr. Weiss has been the principal consultant on this project. Mr. Norman Oslik has also provided considerable consultation in areas of forms and systems design and coordination of data management activities. Ms. Carol Parsons has also assisted in the printing and distribution of forms.

The overall goals of the project are: to demonstrate as widely as possible methods currently available for the detection of breast cancer; to determine if methods for the detection of breast cancer can be applied to the general

population on a large scale; to determine if a negative thermogram is sufficient, under the conditions of this project, to preclude the need for clinical examination and/or mammography for the detection of breast cancer; to evaluate, insofar as is possible, the sensitivity and specificity of thermography, mammography, and physical examination, individually and in combination; to determine if the American Cancer Society can provide a population of women for screening and on a regularly scheduled basis; to characterize the socioeconomic and medical population of women being screened; to study survival and disease status at follow-up in relation to characteristics of the tumor at time of detection; to find out the extent to which women in the project practice breast self-examination and if not, their stated reasons for non-practice; and to investigate risk factors for breast cancers under the conditions of this project.

When fully operational, twenty-nine screening centers in twenty-seven cities throughout the United States will each screen five thousand women the first year of the project and an additional five thousand women the second year. Each of these two groups will then be rescreened annually for four additional consecutive years (approximately one million screenings), and will then be followed for an additional five years by mail or by telephone (approximately one million follow-up contacts). At the present time, each woman is being screened by mammography, thermography, and a physical examination. Cases with findings considered "suspicious" are being rescreened, if appropriate, earlier than the annual examination. Cases with findings considered "abnormal" are being referred to the patients' physicians. Where clinical specimens are taken, data from pathology reports are being obtained. A retrospective case/control study will also be conducted on a subset of the women being screened in order to provide additional information on high risk groups, with a view towards more selective screening. Those women selected for the case/control study will have a more complete history taken by personal interview in the screenee's home. The Comprehensive Patient History (CPH) form will be used to collect data in greater detail on the screenee's family, pregnancy and disease history. Cases will consist of an estimated 4,000 women with positive biopsies. There will be two sets of controls: (1) 4,000 women with negative biopsies; and (2) 4,000 matched non-biopsied women. The case/control study represents a total of approximately 12,000 women who will receive the CPH over the six year screening period.

The ADPMS, in collaboration with Dr. Robert Connor, also of the Biometry Branch, has been instrumental in developing the protocol for the case/control study. A contract has been established to perform all of the interviewing required for this sub-study. Mr. Oslik is a Co-Project Officer on this interviewing contract. He has been involved in all phases of the contractor selection process and has also prepared all materials needed to obtain OMB clearance for the case/control study. Furthermore, Mr. Weiss and Mr. Oslik are consulting with Dr. Connor and Dr. Roger Williams on the planning and operational aspects of a study utilizing the cohort in the Breast Cancer Detection Demonstration Project, to ascertain whether there is a correlation between breast cancer and the use of the drug Reserpine.

Some ten million documents (some several pages in length) will be prepared in the Breast Cancer Detection Demonstration Project. Because of the considerable volume of information being generated, a screenee's complete record will be entered into the computer only for a randomly selected 10% sample of the entire screening population, and for those cases recommended for biopsey. The remainder of the women being screened will have only demographic data and the final recommendation for each of their visits to the screening clinic entered into the computer. Although not all of the data are to be processed by computer, nevertheless, in terms of the data management effort involved, this project is quite complex.

A contract was awarded to the University City Science Center of Philadelphia in fiscal 1973 to establish a Data Management Center to perform all of the data management and computer processing work required in this project. This includes the design, implementation, operation, and maintenance of a computer system, the keying of all appropriate data, the preparation and maintenance of procedures manuals, providing training on forms use and procedures, data purification, storage, retrieval for analysis, quality control of all data management activities, computer facilities, and an office for operational planning, document handling and storage. Mr. Weiss is the Co-Project Officer for this Data Management Center contract.

Copies of all forms are received in the Data Management Center where they are microfilmed, and where the data are transferred to magnetic tape and subjected to a rigorous edit for completeness, accuracy and consistency. Errors, omissions and inconsistencies are reported back to the Clincs for review and the submission of corrections. A separate manual quality control check is conducted periodically to review the accuracy of the data conversion work performed under a "keying" subcontract. Mailing labels and listings are prepared for the clinics to facilitate the scheduling and recall of women for annual re-screening. Stringent security and confidentiality measures are followed at the clinics and the Data Management Center to assure the privacy of each patient.

Monthly Statistical Reports are prepared for each clinic, consisting of the results of each test modality (mammography, thermography and physical examination), the number of biopsies and aspirations recommended, and the number of cancers actually diagnosed as part of the Screening Program. Frequency tables for the descriptive data, which show the makeup of the population being screened, are distributed to each of the screening clinics. Efforts are now underway to provide correlative information relating the patients' demographic characteristics and medical histories to the screening results.

Consultation on this project is expected to continue at a fairly extensive level.

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Prevention Branch

Tyler Texas Asbestos Workers Program

This Program was initiated by the Division of Cancer Control and Rehabilitation in conjunction with the Carcinogenesis Area, DCCP. Mr. Theodore Weiss is serving as the consultant. Investigators from five other independent research institutions are also collaborating on this Program. They are the: East Texas Chest Hospital, Texas Chest Foundation; Mt. Sinai School of Medicine; National Institute of Occupational Safety and Health; University of Texas Southwestern Medical School; M.D. Anderson Hospital and Tumor Clinic; and Baylor University College of Medicine.

In 1972, when the asbestos plant in Tyler Texas was closed after continuous operation from 1954 - 1972, employees of the asbestos plant completed a period of prolonged exposure to inhaled amosite asbestos fibers. As a consequence of this exposure, many of the former employees will develop cancer, mainly bronchogenic carcinoma, and die from this disease unless some type of successful intervention is undertaken. Some of the workers already have, or may be expected to develop mesothelioma, asbestosis, and other asbestos-related respiratory disorders. Furthermore, it is expected that members of the workers' families, others who resided in or visited the plant or workers' homes, those who worked in service establishments handling workers' clothes, etc., workers in commercial gardening firms using quantities of burlap bags purchased from the asbestos plant, and other temporary workers assigned to load/unload asbestos from the railroad cars may also be at an excessive risk. There is also some concern regarding the general population in the geographic area of the asbestos plant.

The project is being undertaken to make available medical and scientific expertise and technology currently in existence or under development, to the asbestos workers, their families, and others who may have been exposed to this carcinogen in Tyler; in order to prevent or lessen the impact of asbestos-related diseases on this group of people through the earliest possible diagnosis of incipient lung cancer; to demonstrate the applicability of state-of-the-art medical, scientific, and technological capabilities and developments to the medical community both in Tyler and elsewhere; and finally, because the above situation represents a unique opportunity; this project will allow us to study the pathogenesis and detection of lung cancer induced by asbestos, in order to further develop and evaluate control measures for the prevention of this disease. Because of the long-term pathogenesis of some of the respiratory diseases being studied here, several aspects of this project may continue for as long as twenty to thirty years.

The primary facility at which this project is being conducted is the East Texas Chest Hospital in Tyler, Texas. This hospital is the main facility of the State of Texas Department of Health for education and research activities in all chest diseases. Former plant employees and a control group are being encouraged to come to the hospital every six months for a two-hour battery of tests, including physical examination, chest x-ray, and sputum cytology.

The specimens for microscopic examination of cells are being obtained from the workers' lungs through deep cough samples. Abnormal cells in the sputum may indicate lung cancers too small to be detected by other methods. Additional procedures include taking comprehensive medical histories, measuring the breathing capacity of each worker's lungs, and collecting blood samples from each individual to be stored for immunologic and biochemical evaluation.

At the present time, several sub-studies involving the Tyler cohort are contemplated by the National Cancer Institure, but these are still in the planning stages. They include: (1) a study of the families of the asbestos workers (and possibly others in the Tyler area who resided in the worker's homes, workers who loaded/unloaded asbestos from railroad cars, employees of commercial gardening firms who used surplus burlap bags from the asbestos plant, non-asbestos worker residents of the Tyler area, and individuals who provided services to the asbestos workers), all of whom may also have had a significant amount of exposure to the asbestos; (2) an intervention trial involving prophalactic pharmacology; and (3) an educational program to alert all individuals who may have had significant exposure to the asbestos in the Tyler area. Furthermore, it is likely that special projects will be initiated during the course of the Tyler Study (e.g., determining the usefulness of certain biological markers, screening for gastrointestinal cancers, etc).

The Tyler Study has been in operation since June 1974. The clinical and laboratory activities were initiated as quickly as possible in order to provide maximum benefits to the workers. A considerable amount of operational work has been performed including locating workers, taking histories, screening subjects and controls, evaluating the results of the examinations, and referring workers in need of treatment to physicians.

Mr. Weiss has been instrumental in planning and establishing a Statistical Data Management and Coordinating Center (SDMCC) for the study. The center is responsible for assuring that enough data are being gathered on each subject; that a complete and proper set of project forms is put into use; that the project itself is well-designed and scientifically sound; that it meets acceptable criteria for statistical validity; that the data gathered show completeness accuracy and consistency; and that preliminary (and eventually final) data are interpreted and disseminated quickly to the investigators participating in the Study. Furthermore, a computerized data management and statistical computing system will be developed under this SDMCC contract in order to support the clinical and office activities in Tyler, maintain the patient registry, store, purify, retrieve and synthesize the large amount of complex data being gathered on each subject, and facilitate the analysis of the Tyler data independently and the comparative analyses of the Tyler data with those from the Paterson cohort and other similar studies. This contract will also provide resources to assist the NCI Project Officers in the planning, evaluation and coordination of all aspects of the Study as it is presently constituted, and as it may evolve over time.

Mr. Weiss prepared the RFP,Contract Workscope, Project Plan, and other material for the SDMCC contract, participated in the selection of the contractor

and in the organization, staffing and start-up of the patient registry. Mr. Weiss also participated in a comprehensive review of the operation in Tyler and he has been working closely with all of the participants in the project to coordinate their activities with the establishment of the Statistical, Data Management and Coordinating Center.

Office of the Director, NCI

Mr. Weiss provided consultation to the National Cancer Program-Management Information System through membership and participation in the MIS Operations Subcommittee. This group is responsible for coordinating technical aspects of the various subsystems in the MIS, implemented for a variety of organizations within the NCI. These aspects include training, documentation, standards, security, procedures, staffing, and subsystem testing.

Mr. Weiss was invited to become a member of the Data Processing Committee of the Association of American Cancer Institutes during the year. He will consult with other committee members of the AACI to upgrade the level of biomedical computing within the institutes through the exchange of methodology and the development of solutions to common problems. Mr. Larson was invited to membership on the Frederick Cancer Research Center EDP Working Group to review the progress and performance of the data processing aspects of the Frederick Cancer Research Center Management and Operation Contract.

Other Collaborative Activities

OSLIK, N.: Member, Grant Application Review Committee, Division of Extramural Affairs, NHLI, University of Pennsylvania Pulmono-Cardiac Center of Excellence;

STUMP, J.M.: Member, Grant Application Review Committee, Division of Cancer Grants, NCI, Community Cancer Center Support, University of Tennessee; Member, Contract Review Committee, Division of Blood Diseases and Resources, NHLI, Statistical Coordinating Center for Sickle Cell and Hemolytic Diseases Cooperative Study Group, Department of Biostatistics, University of North Carolina;

WEISS, T.: Member, Grant Application Review Committee, Division of Extramural Affairs, NHLI, Harvard University, Program Project in Respiratory Mechanics; Member, Contract Review Committee, Division of Heart and Vascular Diseases, NHLI, Central Coordinating Center for Lipid Research Studies, Department of Biostatistics, University of North Carolina; Member, Clinical Trials Review Committee, NHLI, Hebrew University Proposal to Establish a Lipid Research Clinic; Member, Clinical Trials Review Committee, Maryland University, Research and Development of Methods for Clinical Trials.

Significance to Biomedical Research and the Program of the Institute:

The systematic capture, organization, and display of complex and diverse data are of considerable importance in the planning, conduct, and management of the research efforts for the National Cancer Program. As multidisciplinary and collaborative activities increase in scope and complexity, the problems of linking, manipulating, analyzing, and communicating large quantities of complex data and information become unmanageable without the assistance of computer-related technology.

Proposed Course of the Project:

Consultation and technical support will continue to be provided to this Division's research activities. The level of consultation and assistance is expected to increase in other areas of the Institute.

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1975

Office of the Chief

AICHI CANCER CENTER RESEARCH INSTITUTE (NO1CP23213)
KUAKINI HOSPITAL AND HOME (NO1CP33216)

Title: Study of Cancer Among Japanese Migrants

Contractor's Project Director: Professor Mitsuo Segi (NO1CP23213)
Dr. Grant N. Stemmermann (NO1CP33216)

Project Officer (NCI): Mr. William Haenszel

Objectives: The purpose of these contracts is to collect data bearing on the reasons for the differences in cancer incidence between Japanese on the home islands and the Japanese migrant populations in Hawaii and California. The objective is to sort out those aspects of common cancers which may be genetically involved and those which may derive from aspects of the environment or some mixture of the two.

Methods Employed: Standard demographic techniques are employed, basically using a system of diagnosed cases and matched controls. In addition, data have been collected from samples of Japanese households. The matched case-control technique is particularly useful in this kind of study. Pathology protocols are being completed for cases in the study series for several sites (stomach, colon, rectum) and considerable emphasis is being placed on the correlation of the epidemiological and pathology findings. In preparation for later case-control studies of prostate cancer, latent prostate cancer is being studied in 5 groups of patients. A prospective study has been organized at Kuakini Hospital to study a defined cohort of Japanese men in Hawaii.

Major Findings: Study of 179 Japanese patients with bowel cancer and 357 hospital controls in Hawaii revealed excess risks for persons who regularly ate Western-style meals only. The similarity in findings for Issei and Nisei sharply contrasts with results previously reported for stomach cancer in Issei and Nisei, where the reduced risk among persons regularly eating Western-style meals only was limited to the second-generation Nisei. Bowel cancer was associated with consumption of specific foods. Bowel cancer patients ate meats, legumes, and starches more frequently, with the case-control disparity being less pronounced for individuals consuming starches than for those eating meat and legumes. Beef and string beans were the major contributors to the meat and legume effects. There seemed to be separate effects for beef, string beans, and starches, and the case-control contrasts were strengthened when various combinations of foods were considered. These results may narrow the area of search for etiologic factors and thus assist in planning future research on the complex sequence of events leading to bowel cancer.

The cancer risks for Japanese migrants differ from those observed in Japan; the migrant risks are higher for lung, colon, rectum, prostate and lower for stomach while little change has been observed for breast among post-menopausa women. The case-control studies show an excess consumption of salted and dried fish and pickled "salted" vegetables some of which are rich in secondary amines, by stomach cancer patients. Since nitrites and secondary amines can be synthesized in vivo into nitrosamines, potent carcinogens, a possible etiology is suggested for further study. Attempts will be made to confirm these case-control findings using the prospective aspects of the cohort study in Hawaii.

Latent prostate cancer is almost as common in Japanese as in Americans though Japanese have little clinical cancer of this site. A possible resolution of this anomaly has been contributed by Dr. Akazaki, who has described and define two types of latent carcinoma - proliferative and non-proliferative. The gradient in prevalence of the proliferative type lesion in his autopsy studies of specimens from Japan and Hawaii Japanese corresponds closely to the gradient in incidence of clinical prostatic carcinoma in the two populations. The important feature of the Hawaii Japanese males is the greater amount of proliferation and not their unremarkable overall prevalence of occult lesions, which suggests the presence of some promoting factor in the migrant male population.

Significance to NCI Program and Biomedical Research: If the studies in these migrant populations elucidate the environmental factors involved in the incidence of certain forms of cancer, and indicate those that are predominately environmental and those which are predominantly genetic, then it may be possible to apply these findings to the United States population for purposes of cancer prevention and cancer control.

Proposed Course: These two contracts are expected to continue for several years because of the long range nature of these studies. The male cohort of Japanese being followed at Kuakini is being expanded to include brothers and may be further expanded to include sisters, wives and daughters for possible breast cancer studies.

Date Contract Initiated: February 1972 (NO1CP23213)
June 1971 (NO1CP33216)

Current Annual Level: $154,000 (NO1CP23213)
$497,876 (NO1CP33216)

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1975

Office of the Chief

AMERICAN CANCER SOCIETY (NO1-CP-43213)

Title: Study of Smoking and Mortality Among Veterans

Contractor's Project Director: Dr. E. Cuyler Hammond

Project Officer (NCI): Mr. William Haenszel

Objectives: This project is for the final collection of data for the study of smoking and mortality among U.S. Veterans started by Dr. Harold Dorn in 1954. The original cohort was comprised of 293,658 veterans who held U.S. Government Life Insurance policies. A great amount of data has been collected on these people, but the information from 32,000 death certificates since 1962 still remains to be obtained and coded.

Methods Employed: Dr. Hammond of the American Cancer Society will obtain and code these 32,000 death certificates in conjunction with the current mortality follow-up of his smoking study cohort of over one million persons. He estimates that 25,000 of these records can be obtained routinely, but that the rest will require a search in various health departments by ACS field personnel.

Major Findings: Information from death certificates is still being collected and no results are available yet.

Significance to NCI Program and Biomedical Research: This contract will allow completion of a major long term study on smoking and health, and will result in a series of important papers on this cohort, the ACS cohort and a cohort of English physicians.

Proposed Course of Project: This study is expected to be completed by April 1976.

Date Contract Initiated: July 1, 1973

Current Annual Level: $66,000

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1975

OFFICE OF THE CHIEF

CALIFORNIA STATE DEPARTMENT OF HEALTH (NO1-CP-33353)
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO (NO1-CP-43383)
CHARITY HOSPITAL OF LOUISIANA (NO1-CP-43262)
CONNECTICUT STATE DEPARTMENT OF HEALTH (NO1-CP-43203)
FRED HUTCHINSON CANCER RESEARCH CENTER, SEATTLE (NO1-CP-33246)
RESEARCH CORP. OF THE UNIVERSITY OF HAWAII (NO1-CP-53511)
UNIVERSITY OF IOWA (NO1-CP-43200)
MICHIGAN CANCER FOUNDATION (NO1-CP-43264)
UNIVERSITY OF NEW MEXICO (NO1-CP-33344)
UNIVERSITY OF UTAH (NO1-CP-43382)
YALE UNIVERSITY (NO1-CP-33235)

Title: Surveillance, Epidemiology, and End Results Reporting (SEER) Program

Project Officer (NCI): Sidney J. Cutler, Sc.D.

Objectives: To obtain and analyze data on morbidity, mortality, and survival from population-based registries, to identify areas for epidemiologic investigations, and to initiate preliminary investigations needed to develop epidemiological study protocols.

Methods Employed: The National Cancer Institute is sponsoring a collaborative program for Cancer Surveillance, Epidemiology, and End Results Reporting (SEER Program). Participants in this program are all population-based registries, covering entire states or geographically specified areas, usually Standard Metropolitan Statistical Areas. The Biometry Branch has professional and technical responsibility for supervising these contracts. Basic information is collected on all patients treated for cancer in the area, area residents treated outside the area, as well as anyone dying in the area where cancer is mentioned anywhere on the death certificate -- or area residents dying outside the area, where cancer is mentioned anywhere on the death certificate. Periodic follow-up information is obtained on the vital status of all those in the registries or from a complete geographic subsample. Data are submitted annually to NCI on uniformly formated computer tapes. These data are available for analysis as well as for providing an extensive resource for special studies.

Core epidemiological staff are present in most of the SEER programs. These staff members will review the morbidity data to identify epidemiological research leads that should be investigated. This staff will develop the study protocol and field test the study schedules. At this point, the program would request separate funding from NCI, either through contract or grant mechanism, or seek financial assistance from other sources.

Significance to NCI Program and Biomedical Research: Information on incidence, survival, and mortality from a representative cross section of the U.S. population is required on a continuing basis so that the nature and magnitude of the cancer problem including changes over time can be determined. Through close scrutiny of variation in cancer incidence and survival with respect to geographic and demographic characteristics of the population and differential changes over time, specific etiologic hypotheses will emerge which, when tested via special study mechanisms, should then lead to the identification of controllable risk factors. Such research is an integral part of the ultimate goal of the national program: the reduction in the occurrence of and mortality due to cancer. The SEER Program would also serve in the evaluation of cancer control programs conducted in the areas covered by the registries. They would also provide the mechanism for identifying patients who had suspicious or positive findings on screening tests but were either not followed for any extended period of time, or were lost to follow-up and were eventually diagnosed with a malignant neoplasm. A system of record linkage between the cancer registry and employees of specific industries or specific types of occupations would provide early warning of possible occupational carcinogenicity. This would significantly reduce the time when cancer hazards would be identified.

Major Findings: The SEER Program is a new venture. Data on morbidity from all areas was received by NCI for the year 1973 by the end of 1974. It will require a number of years of checking and evaluation to insure that all areas are submitting accurate, complete, and comparable data. A continuing series of reports will be generated once the above has been completed.

Proposed Course of Projects: Emphasis in the next few years will be on setting up and maintaining a system of quality control to insure 1) complete reporting of all cases in the specified geographic area covered by the registry, and 2) a high degree of accuracy and completeness for the coded information submitted to NCI. Training programs will be developed and carried out for the purpose of instructing field staffs on new codes and procedures and to reinforce present operational procedures.

Special studies will be conducted on various methods of obtaining follow-up information such as physician and patient contact, or death record clearance to determine the effectiveness of the method and costs involved.

The new coding scheme for specifying extent of disease prior to therapy and diagnostic procedures for cancers of the breast and colon/rectum will be implemented and evaluated. The evaluation will determine the accuracy, completeness and comparability of the schemes in the work situation, as well as the costs involved in abstracting time. New schemes for cancers of the cervix, corpus, stomach, prostate, and lung will be field tested and completed. Workshops will be conducted prior to implementing these new schemes.

Site-specific detailed therapy codes will be developed for the same sites for which there are expanded extent of disease schemes. This will assure more specific analyses on correlations between extent of disease and therapy, as well as evaluation of diagnostic procedures and therapy.

	Year Started	FY-75 Obligation
California State Department of Health (NO1-CP-33353)	1962	$894,000
University of California, San Francisco (NO1-CP-43383)	1974	90,000
Charity Hospital of Louisiana (NO1-CP-43262)	1973	198,021
Connecticut State Department of Health (NO1-CP-43203)	1963	277,459
Fred Hutchinson Cancer Research Center (NO1-CP-33246)	1973	350,000
Research Corp. of the University of Hawaii (NO1-CP-53511)	1971	454,193
University of Iowa (NO1-CP-43200)	1973	379,507
Michigan Cancer Foundation (NO1-CP-43264)	1973	468,842
University of New Mexico (NO1-CP-33344)	1973	347,632
University of Utah (NO1-CP-43382)	1973	231,149
Yale University (NO1-CP-33235)	1973	313,826

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
FISCAL YEAR 1975

Office of the Chief

HEALTH INSURANCE PLAN OF GREATER NEW YORK (NO1-CP-43278)

Title: Evaluation of Periodic Breast Cancer Screening by Mammography

Contractor's Project Director: Dr. Raymond Fink

Project Officer (NCI): Dr. Sidney Cutler

Objectives: A. To determine whether a simple screening technique (x-ray mammography) can diagnose breast cancer early.

B. To determine if the very early diagnosis of breast cancer is really useful in improving long-term survival.

C. To investigate through a prospective study of the women screened, the relationship of a wide range of parameters to the development of breast cancer.

Methods Employed: This is a combination statistical-epidemiological-medical study in which a study population receiving mammography at annual intervals and a control group of 30,000 women each are being routinely followed for breast cancer experience through internal Health Insurance Plan files and through review of death certificates. Details of the techniques are given in a paper describing the methodology and initial findings of this study entitled, "Evaluation of Periodic Breast Cancer Screening with Mammography" by Sam Shapiro, Philip Strax, and Louis Venet, JAMA 195: 731-738, 1966.

During one reexamination cycle 40 cc of blood was taken from women in the study population and stored.

Major Findings: Findings to date strongly suggest the usefulness of annual screening which includes mammography. Over the short run period of 6 years of follow-up, the study group of women have about one-third less mortality from breast cancer than those in the control group. The differential between control and study cases in fatality from breast cancer is almost entirely due to the exceptionally low rate among the cases detected through screening. Both the clinical examination and mammography contributed to this favorable situation but mammography, with a fatality rate of only 2% among cases detected in the absence of positive clinical findings during screening, was especially important.

In examining various risk factors for the women who appeared for an initial screening the following women were found to have a 20-30% increase in risk of breast cancer: higher educated women, Jewish women, and unmarried women. Increasing age at first pregnancy, low gravidity, and menarche at ages under 15 are associated with a 50-100% increase in risk.

Significance to NCI Program and Biomedical Research: Early diagnostic procedures may be of use in the potential cure of cancer patients. It appears that mammography is such a technique, and is being extended to other populations.

Proposed Course of Project: The screening part of the study is complete and the emphasis from now on will be on follow-up. The women will be followed for as long a time period as necessary to demonstrate whether the technique is truly useful as an early diagnostic device with long term impact on mortality from breast cancer. The duration of the study is contingent upon the answers to the questions implied by the objectives.

Supplemental lines of inquiry may be suggested by the Breast Cancer Task Force. Proposals for use of the stored blood plasma are being developed.

Date Current Contract Initiated: November 1, 1962

Current Annual Level: $131,399

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1975

Office of the Chief

ISRAEL CENTER FOR REGISTRATION OF CANCER AND ALLIED DISEASES (NO1-CP-33351)

Title: Cancer Incidence Study and Registry Assessment

Contractor's Project Director: Dr. Ruth Steinitz

Project Officer (NCI): Dr. Sidney J. Cutler

Objectives: The first two years of the planned three year project were devoted to collection of cancer incidence data so that a report at the end of the third year would analyze 12 years of cancer incidence (1960-71) for the entire population of Israel. Part of the objectives concerned assessment and resulting changes in the data collecting and processing systems in order to produce epidemiologic data more quickly.

Major Findings: Changes in personnel practice and in technology have increased the efficiency of the organization. An interim planned analysis was presented by the Project Director at the Cancer Congress in Florence in 1974 which contrasted cancer incidence by site for two five-year periods, 1960-64 and 1965-69. Warfare during the contract period delayed the release of official census data and, as a result, the computations required for the 12-year report. A nine month contract extension permits change in the period covered to 1961-1972 to make use of both the 1961 and 1972 censuses. The final report will emphasize epidemiologic findings and suggest studies based upon such findings.

Significance to NCI Program and Biomedical Research: Because of the large number of ethnic and immigrant groups, Israel's cancer data have unique epidemiologic potential and fit well into the Branch's collaborative program concerning studies of change in cancer incidence and mortality after migration. Of major interest is the fact that specific differences in cancer incidence between population subgroups are reported from Israel which far exceed those found between different population groups in the U.S. Data from the population based Israel Cancer Registry can also be usefully integrated in analyses of data generated by the Branch's SEER Program which is also population based.

Proposed Course: The final report will receive critical review to establish whether the findings and the proposed epidemiologic studies merit continued NCI financial support of the data collection system and/or specific studies.

Date Contract Initiated: June 28, 1972

Current Contract Level: $30,000

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1975

Office of the Chief

LOUISIANA STATE UNIVERSITY MEDICAL CENTER (NO1-CP-53521)

Title: Studies of Epidemiology and Geographic Pathology of Cancer

Contractor's Project Director: Dr. Pelayo Correa

Project Officer (NCI): Dr. James L. Murray

Objectives: The purpose of this project is to continue the present research on premalignant lesions in the large intestine being done by Dr. Jack Strong and to develop related cooperative studies with investigators in various Latin American countries. Dr. Pelayo Correa joined Dr. Strong in May and has become the Principal Investigator on this project. He will expand the cooperative research efforts with investigators in Colombia on stomach, prostate, breast and cervical cancer. New pathology case series will be started in New Orleans on prostate, pancreas and breast cancer for future comparative purposes.

Methods Employed: Lesions are identified by a careful gross and microscopic study of bowel specimens obtained at autopsy. In order to develop a new general program of research in cancer epidemiology and geographic pathology in Latin America, special centers or groups of university affiliated researchers with competence, capability and interest are being identified and contacted.

Major Findings: The collection of colon specimens from autopsies in New Orleans is continuing as planned and approximately 1,100 cases will have been collected by the end of 1975. The prevalence of adenomatous polyps will be calculated by age, sex, race and anatomic location within the large intestine. Dr. Correa is expanding the cooperative research efforts with investigators in Colombia on stomach, prostate, breast and cervical cancer. He has already developed cooperative studies on stomach and colon cancer in Brazil and Costa Rica, and new research is being planned in Puerto Rico, Mexico, Paraguay, Bolivia and Peru.

Significance to NCI Program and Biomedical Research: It is hoped to identify those epithelial lesions which are epidemiologically related to colon cancer. This will not only provide clues on the pathogenesis but will identify individuals at high risk for colon cancer and provide a signal of exposure closer in time to the contact of patient and environmental factors. The cooperative studies in Latin American countries offer unique opportunities to identify important factors associated with cancer etiology.

Proposed Course: This contract will continue for several years and will serve as a focal point for collaborative studies in Latin America.

Date Contract Initiated: January 1, 1975

Current Annual Level: $95,600

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1975

Office of the Chief

NORWEGIAN PUBLIC HEALTH SERVICE (NO1-CP-43247)
UNIVERSITY OF MINNESOTA (NO1-CP-33325)

Title: Incidence, Prevalence and Mortality from Cancer in Selected Migrant Populations (Norwegian)

Contractor's Project Director: Dr. Einar Pedersen (NO1-CP-43247)
Dr. Leonard Schuman (NO1-CP-33325)

Project Officer (NCI): Mr. William Haenszel

Objectives: To determine the reasons for difference in cancer incidence and mortality in sedente populations and migrant populations of the same ethnic and genetic composition.

Methods Employed: Standard epidemiologic and demographic methods including prospective observations on defined cohorts and case-control studies.

Major Findings: The University of Minnesota has defined a cohort of approximately 20,000 policyholders in an insurance company that has a high proportion of persons of Norwegian descent and will monitor the group prospectively for mortality data. The contractor has also carried out case-control interviews for gastrointestinal cancer among persons of Norwegian descent in Minnesota and is prepared to extend field work to other sites at a later date. All phases of the cohort and case-control studies in Minnesota are closely coordinated with the ongoing work in Norway.

The introduction of a personnel identification number system in Norway has made record linkage a simple operation, made a large number of studies possible, and facilitated keeping track of cohorts used in the migrant studies. The personnel identification number system is being used to link current study records with data from the 1960 Norwegian census to better identify the characteristics of various population groups. This enabled a study of nickel workers to be accomplished at a much lower cost than would be possible in the United States of America.

The Norwegian Registry also provides one of the means for doing ad hoc international studies. For example, there is interest in studying the effect of differences in incidence rates for such sites as breast and large bowel on the stage at diagnosis and the survival rates. The studies are done more effectively using different countries for study populations. A comprehensive report on patient survival for all cancer cases diagnosed in Norway between 1953 and 1967 is being readied for publication.

Significance to NCI Program and Biomedical Research: This is one of a group of migrant studies to determine those elements in the incidence and mortality from cancer which may be related to environment and those which may be related to genetic factors. Isolation of environmental elements may be useful in cancer prevention within the United States.

Proposed Course of Project: This project in Norway and Minnesota will continue for several more years. The basic migrant study will continue and analysis of the data on respiratory cancers and gastrointestinal cancers will be accelerated. New studies may also be started on breast cancer, thyroid cancer and Hodgkin's disease.

Date Contract Initiated: March 1964 (NO1-CP-43247)
June 1966 (NO1-CP-33325)

Current Annual Level: $95,000
$20,715

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1975

Office of the Chief

UNIVERSIDAD DEL VALLE -- FUNDACION PARA LA EDUCACION SUPERIOR (NO1-CP-33286)

Title: Epidemiology-pathology studies of cancer in Colombia

Contractor's Project Director: Dr. Carlos Cuello

Project Officer (NCI): Mr. William Haenszel

Objectives: To determine site-specific cancer incidence in Cali and other cities in Colombia, to correlate the morbidity data with pathology findings, and to conduct epidemiological studies of specific sites based on leads suggested by these data.

Methods Employed: The cancer morbidity surveys in Cali will be continued with emphasis on variation by socioeconomic class and place of birth. Internal migration within Colombia is of such magnitude that two-thirds of the residents of Cali were born elsewhere. Cancer morbidity surveys of a limited scale and duration will be conducted in Cartegena and Medellin, cities thought to have greatly different site-specific incidence.

In Cali an organ bank of tissues from persons dying from accidents and acute diseases is being utilized for study of occult tumors and malignant precursors with emphasis on stomach, colon and cervix. The organ bank findings also emphasize the distribution by place of birth and are used to complement the incidence data from the morbidity surveys. Similar pathologic material is being collected in New Orleans and Hawaii. Autopsy studies directed to suspect precursor lesions of cancer of the stomach (intestinal metaplasia) and of the large bowel (polyps) are being conducted in Cali, Medellin, San José (Costa Rica) and São Paulo (Brazil).

Population screening, case-control studies of diet and characterization of suspect environmental factors are underway in the department of Nariño, an area presenting very high stomach cancer risks.

Major Findings: The Cancer Registry of Cali has assembled the incidence data for the 10-years 1963-72 for publication in the 3rd edition of Cancer in Five Continents. Special studies are continuing on the precise localization of large bowel cancer lesions and their histologic characteristics. Cali is at low risk to bowel cancer, much of which is accounted for by a deficit of tumors in the sigmoid colon. Cali has been shown to have a strong social class gradient in colon cancer (a feature not seen in North America and Western Europe) with the upper classes having risks 2 1/2 times those of the lower classes. The social class differences in anatomical localization of large bowel tumors mimic those found in contrasts of high- and low-risk countries.

Studies of adenomatous polyps in a Cali and San José have also demonstrated these populations to have the low prevalence characteristic of populations at low risk to large bowel cancer.

The comprehensive epidemiologic study of stomach cancer being conducted, in the Department of Nariño has revealed strong clustering of cases that may be associated with nitrates in water supplies and selected food items. The observational situation is very favorable for pursuit of diet and stomach cancer relationships. Studies of lymphoreticular tumors are continuing and are directed toward elucidation of environmental and host factors. Some cooperative efforts with the International Union Against Cancer (UICC) have been developed. The collaborative study of thyroid cancer with investigators in Hawaii has been completed. Occult carcinomas were found in 5.6% of the autopsy samples which is similar to that found in the United States. A similar collaborative study on prostate cancer is being conducted with investigators in Japan to assess the frequency of occult cancerous lesions in the male population. A histologic study of cervical cancer is underway in Cali and a mixed cell type tumor is often seen which does not fit into the usual Reagan classification system.

Significance to NCI Program and Biomedical Research: This contract is producing significant data for comparative studies in the United States and elsewhere, with emphasis on areas providing the greatest contrasts in risks for specific cancer sites.

Proposed Course of Project: The various aspects of this project will continue for several years. Emphasis will be placed on clinical and experimental studies of the genesis of intestinal metaplasia and carcinoma of the stomach. New collaborative research efforts will begin with investigators in Brazil and Costa Rica.

Date Contract Initiated: June 29, 1966.

Current Annual Level: $98,684

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1975

ADP Management Section

WOLF RESEARCH AND DEVELOPMENT CORP. (NO1 CP 33254)

Title: Computer Programming Services for the Third National Cancer Survey

Contractor's Project Director: Edwin Lisiecki

Project Officer (NCI): James E. Larson

Objectives: This contract provided computer software support to the Third National Cancer Survey until March 31, 1975.

Major Findings: Work on this contract during the current year was primarily in support of the Demography Section's final data base clean up and report generation for activities for the publication of NCI Monograph No. 41 (Third National Cancer Survey; Incidence Data). This publication of over 450 pages of statistical tables was produced using the Government Printing office's computer-driven type setting equipment "Linotron". Wolf prepared all of the necessary computer programs and coordinated the entire effort.

Wolf's staff also provided considerable systems analysis and computer programming to numerous special studies of the TNCS data. These studies included the analysis of incidence by socioeconomic status, by county of residence, by marital status, and by urban or rural place of residence at diagnosis. Wolf also developed and operated a computer system to process and analyze Ultra Violet Light Radiation Exposure data.

Significance to Biomedical Research and the Program of the Institute:
See Projects Reports, Demography Section, Biometry Branch, NCI.

Proposed Course: Work on this contract was completed on March 31, 1975.

Date Contract Initiated: February 11, 1973

Final Contract Level: $290,757

CONTRACT NARRATIVE
BIOMETRY BRANCH, DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1975

Clinical and Diagnostic Trials Section

MASON RESEARCH INSTITUTE (NO1CP-53524)

Title: Computer Services

Contractor's Project Director: Anne Kramer

Project Officer (NCI): Dr. David P. Byar

Objectives: This program provides systems design and computer programming support for projects in the Clinical and Diagnostic Trials Section.

Major Findings: During the past year the contractor has maintained a Veterans Administration Cooperative Urological Research Group (VACURG) computer system comprised of approximately 80 computer programs which edits new data, updates master files, and produces a semi-annual report containing numerous tables and other data displays. The report for November 1974 consisted of 197 pages of extensive tabulations of data. The results are derived from complex statistical analyses, sophisticated data handling and analytical techniques, and data graphically plotted by digital computer. Since important clinical decisions are based on this report, up-to-date data must be presented as quickly as possible. Much of the computer output, therefore, must be in a form suitable for direct photographic copying. Before the report can be prepared, the data bases of five major clinical trials are updated with follow-up information received since the last report, and then submitted for a thorough computer editing.

Wolf personnel have written numerous computer programs for special studies related to the VACURG study and for other selected studies in which the Section participates. This often involves developing computer programs to facilitate the study of properties of medical and demographic data utilizing complex analytical techniques and decision rules. One such study involved the analysis of tumor histological data. This data represents some 3000 patients participating in the prostate studies. Programs were written to edit and update this data file. Since no previous attempt had been made to edit this data, completion of this task required extensive personal communication with the pathologist. Programs were written to combine histological data with clinical, biochemical and mortality data for these patients. Analytical programs were written under the direction of the Project Officer and the correlation between tumor histological type and patient survival was verified.

In addition, an instruction manual was written to facilitate the editing and updating of the data files and the generation of the semi-annual report. The manual describes the steps and programs used to accomplish the above.

Proposed Course: During fiscal year 1975 this contract was extented for a further year and is currently scheduled to be in operation through the end of March 1976. The name of the contractor was changed due to reorganization to Mason Research Institute.

Date Contract Initiated: February 28, 1972

Current Contract Level: $93,058

CONTRACT NARRATIVE
BIOMETRY BRANCH, OADFS&S
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1975

Demography Section

UNIVERSITY OF ALABAMA MEDICAL CENTER (Birmingham) (NO1-CP-90049)
COLORADO DEPARTMENT OF PUBLIC HEALTH (Denver) (NO1-CP-90050)
MEDICAL ASSOCIATION OF GEORGIA (Atlanta) (NO1-CP-90047)
UNIVERSITY OF MINNESOTA, SCHOOL OF PUBLIC HEALTH (Minneapolis) (NO1-CP-90045)
UNIVERSITY OF PITTSBURGH (NO1-CP-90043)
UNIVERSITY OF TEXAS, SOUTHWESTERN MEDICAL (NO1-CP-90040)

Title: Third National Cancer Survey

Contractor's Project Director: See table on following page

Project Officer (NCI): Sidney J. Cutler, Sc.D.

Objectives: Procurement and initial processing of data for the Third National Cancer Survey

Major Findings: An NCI monograph containing incidence data by site and type of cancer for all areas combined and for each area separately is in press at the Government Printing Officer. For specific findings see Individual Project report NCI-4268.

Significance to Biomedical Research and the Program of the Institute: See Individual Project Report NCI-4268.

Proposed Course: All data collection has been completed. Three field offices have been absorbed into ongoing registry programs which will participate in the NCI SEER Program (see Individual Project report NCI-4254). All other field offices were closed by August 31, 1974. These contracts have now expired.

Date Contract Initiated: See table on following page.

Current Contract Level: See table on following page.

Contractor	Director	Initiated	Period	Level
University of Alabama	Dr. Harold Schnaper	10/1/68	7/1/74-8/31/74	$5,893
Colorado Department of Public Health	Dr. Vernon Wohlauer	10/1/68	7/1/74-6/30/74	-
Medical Association of Georgia	Dr. Gordon Barrow	10/1/68	7/1/74-6/30/74	4,767
University of Minnesota	Dr. Leonard Schuman	10/1/68	7/1/74-8/31/74	-
University of Pittsburgh	Dr. Carol Redmond	10/9/68	7/1/74-8/31/74	-
University of Texas	Dr. Eugene Frenkel	10/1/68	7/1/74-8/31/74	-

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1975

END RESULTS SECTION

NEW YORK MEDICAL COLLEGE (CP3-3321)

SASKATCHEWAN, CANADA (REGINA) (CP3-3350)

Title: The increasing incidence of breast cancer in the Province of Saskatchewan, Canada

Project Officer(NCI): Dr. Benjamin F. Hankey

Objectives: To obtain and analyze data on breast cancer in the Province of Saskatchewan, Canada in order to study whether the increasing incidence rate of breast cancer since 1946 is due to changes in diagnostic criteria and/or changes in the age distribution of women. Other studies were also carried out utilizing the data collected for this study.

Methods Employed: In order to determine to what extent the increase in the incidence rate was due to changes in diagnostic criteria it was necessary to obtain samples of both benign and malignant lesions from various years since 1946. The method of sampling was as follows. In each of four calendar years (1946, 1951, 1960, 1968) clinical data including survival were obtained for the first 100 women seen in each of the two breast clinics serving the Province. These women had been diagnosed by Saskatchewan pathologists as having either benign or malignant lesions. All slides used for diagnostic purposes were available for these patients and were sent to New York Medical College where they were read by Dr. Maurice Black for diagnostic purposes and also to determine various histologic characteristics of the primary tumor and lymph nodes. Those women diagnosed as having breast cancer in each of the four calendar years by Saskatchewan pathologists were appropriate samples of the breast cancer experience in those years. In order to get appropriate samples of benign lesions it was necessary to go to five hospitals in the Province whose benign lesion experience likely represented at least 90% of the total experience in the Province. Samples of benign lesions were obtained for the same calendar years (approximately 100 in each year) as for malignant lesions and all diagnostic slides for these lesions were read by Dr. Black.

Significance to NCI program and Bio-Medical Research: In-depth investigations of trends in incidence and survival are necessary in order to gain some understanding as to what extent they are artifactual or real. Further examination of such trends with respect to geographic and demographic characteristics of the population should lead to specific etiologic hypotheses which can be tested via the special study mechanism. Such studies should lead to the identification of controllable risk factors which could, in turn, lead to a reduction in the incidence of cancer; an integral part of the ultimate goal of the National Program.

Major Findings: The crude incidence rate of breast cancer in Saskatchewan has increased from 36 per 100,000 in 1946 to 69 per 100,000 in 1972. Taking the 1946 female population as a standard, the age adjusted incidence rate for 1972 is 53 per 100,000. The shift in the age distribution, therefore, explains about half of the increase. Reading the slides originally used by Saskatchewan pathologists for purposes of diagnosis for the samples of both benign and malignant lesions did not indicate any substantial differences between the diagnoses made by Saskatchewan pathologists and those made by Dr. Black. It would not appear that the increase in the incidence rate of breast cancer is due to improved case finding or changes in diagnostic criteria.

Proposed Course of Project: All work related to the original objectives of the contract is essentially completed and current plans call for terminating both contracts this year although they may be administratively extended for short periods after their termination dates. The reason for the possible extension is to permit the collection of some additional data which would permit the further consideration of some other questions which we have addressed utilizing the Saskatchewan registry.

	Date Initiated	FY-75 Negotiated
NEW YORK MEDICAL COLLEGE (CP3-3321)	6-24-71	—
SASKATCHEWAN, CANADA (REGINA) (CP3-3350)	6-21-72	—

SUMMARY REPORT
EPIDEMIOLOGY BRANCH
JULY 1, 1974 THROUGH JUNE 30, 1975

The objective of the Epidemiology Branch is to test ideas concerning the origins of cancer through epidemiologic studies based on medical knowledge and experience and on an awareness of resources best available at the national or international level. An idea is developed from existing knowledge, which may come from the laboratory, the clinic, or other epidemiologic studies. Emphasis is placed on epidemiologic research based on important new information from laboratory investigations at the National Cancer Institute and elsewhere.

Staff Changes: On July 1, Dr. William J. Blot, biostatistician, joined the staff, Dr. John J. Mulvihill returned to Bethesda from Johns Hopkins Hospital where he completed 2 years of clinical training in Pediatrics, and Dr. John J. Chabalko, upon completion of his 2 years as a Staff Associate, became a Resident in Internal Medicine at the Cleveland Clinic.

Organizational Changes: Reorganization of the Branch, the first since it was established more than 14 years ago, brings up to date its functional components and recognizes the leadership provided in them. Dr. Joseph F. Fraumeni, Jr. was appointed Associate Chief of the Branch, Dr. Robert N. Hoover as the Head of Environmental Studies Section, Dr. John J. Mulvihill as the Head of the Clinical Genetics Section, and Dr. Frederick P. Li as Head of the Boston Field Section. Dr. William A. Priester continues as the Head of the Epizoology Section, and Dr. Robert W. Miller is Acting Head of the Special Cancer Studies Section.

Research Program:

Atomic Bomb Casualty Commission (ABCC):

In 1972 a contract was made with NAS-NRC, which is administratively responsible for ABCC, to fill a financial gap caused by devaluation of the dollar -- an event which threatened the closure of ABCC before important further information on radiogenic cancer could be obtained. The contract provided funds for a core cancer program (i.e., the salaries of 43 staff members engaged in cancer studies) and for 2 new projects: Tumor Tissue Registries to be established in each city through subcontracts with the local medical associations, and a Visiting Scientist Program that would bring 2-4 young Japanese faculty members to the U.S. for 2 months for training in cancer epidemiology (usually at the summer session at the University of Minnesota), with the balance of the year to be spent in research at ABCC. In this way it was expected that scientists at the universities would become familiar with ABCC as a resource and with cancer epidemiology as an area for research. The program has attracted seven good young medical scientists and one statistician.

With time there has been severe erosion of the percentage of autopsies performed at ABCC. The biopsy program there has long been defunct. In

consequence, cancer morbidity is not monitored at all; mortality is known to the extent that death certificates are accurate (good for cancer of any type, variable for cancer of specific types). The Registries provide surveillance of cancer morbidity which far exceeds ABCC's capability. The cost, $50,000 annually in each city, is an order of magnitude below that for registries in the U.S. The promise for useful new information about occupational or radiogenic cancers is substantial in Hiroshima and Nagasaki. Interest in using the data seemed greater in Nagasaki, but its availability in both cities fills an important gap in cancer surveillance which should be of great value to the Radiation Effects Research Foundation, the successor to ABCC as of April 1, 1975.

International Union Against Cancer (UICC):

Under the aegis of the UICC's Committee on Cancer in Children, the Epidemiology Branch of NCI continues to collect by mail data from pediatric centers throughout the world for an international comparison of childhood cancer according to cell type. The purpose is to determine if the relative frequencies of certain childhood cancers are inordinately high or low in specific locales. The response has been most generous. More than 60 centers have contributed abstracts on series of patients, giving the diagnoses by cell type. More than 40,000 abstracts have been accessioned. Each contributor receives within a week a tabulation of his data according to a standard format, with comments as to how the relative frequencies of cancers that he has seen differ from the experience elsewhere. In this way he can identify areas of special research interest within his own locale and publish his findings as he sees fit. When unusual occurrences of tumors of specific types were noted in a particular center, histologic specimens were requested for review under an NCI contract with the Albany Medical College. A summary report of the findings from all centers was presented at the XI International Cancer Congress in Italy in October 1974.

Other International Activities:

At the XI International Cancer Congress in Florence, Italy, one member of the Branch served as Chairman of a 2-day Conference on Environmental Factors in Cancer Induction: Appraisal of Epidemiological Evidence (33, 39), another Branch member chaired a Panel on Genetic Factors in Cancer. Our staff presented papers on occupational causes of lung cancer, industrial bladder cancer in the U.S., and an international comparison of childhood cancer by cell type.

One Branch member served on the Epidemiology Working Group of the US-USSR Subcommittee on Research in Oncologic Disease, and another served on the US-Japan Panel on Environmental Mutagenesis and Carcinogenesis (US-Japan Cooperative Medical Science Program) and on the new US-Japan Cooperative Cancer Research Program.

Dr. Masaharu Hitosugi, an epidemiologist at Kitasato University, spent 6 months in the Branch under the sponsorship of the US-Japan Panel mentioned above. He is interested in our approach to cancer epidemiology especially as it pertains to occupational exposures, and hopes to develop a Japanese

counterpart to our county-map system for studies of cancer mortality. Dr. Keiko Omi, a pediatric oncologist at Tokyo University, spent 2 months in the Branch, observing activities concerning the bedside approach to the etiology of childhood cancer (e.g., associations with cancer or other diseases in the same patient or his family).

Environmental Studies:

At a time when it seemed that little more could be learned about carcinogenesis from national mortality data, a large new area was opened by the Branch through its studies of site-specific cancer deaths, 1950-1969, according to county. Maps were created from data on more than 6 million persons with death-certificate diagnoses of cancer in the 20-year interval. An atlas of maps in color showing counties according to their site-specific cancer rates is to be published in May 1975 (28).

The data serve to generate hypotheses (20, 27, 68, 69) (e.g., high urinary bladder cancer mortality among white males related to the manufacture of heavy machinery) and to test hypotheses (e.g., the [lack of a] relation between 1) cancer and asbestos in the Duluth, Minnesota water-supply (29), 2) cosmic radiation at high altitudes (30), and 3) chemical carcinogenesis in drinking water from the Mississippi River).

The atlas should lead to identification of specific high-risk counties -- among the 3056 counties in the U.S., where special searches can be made for previously unrecognized occupational carcinogens. One such instance has already come to light in this way: an epidemic of urinary bladder cancer, well known to the management of a chemical factory in Salem County, New Jersey, was for the first time brought to the notice of local and state health officials.

Occupational studies would be greatly facilitated by the establishment of a National Death Index (NDI), i.e., a central listing of identifying information for each person who dies in the U.S. One could then match employment rosters against the NDI, and when matches were found, the place and date of death could be revealed -- information that would direct epidemiologists to the appropriate state health department to seek in the usual way a copy of the death certificate from which the cause of death would be obtained. The NDI would drastically reduce the time, cost, effort and invasion of privacy in conducting such studies as compared with present methods. The NDI was first formally proposed in 1968. Recently momentum was gained when the General Counsel stated that the concept was not in violation of current laws or regulations. The Branch has been active in the past in trying to establish the NDI because it would so greatly simplify follow-up studies.

A comprehensive review of chemicals that cause lung cancer (78) revealed that only 1 of 12 agents known to induce such neoplasia was first revealed by animal experimentation. The other 11 were found through studies of man, and 10 proved also to be carcinogenic in laboratory animals, the exception being arsenic.

The Branch continues to be among the national leaders in its activities concerning radiogenic cancer -- in supporting research at ABCC, in testing hypotheses about radiation exposures (24, 25, 30) and in interpreting the subject generally (34, 38, 40).

The Branch also is among the leaders in studies of cancer related to immunosuppression, either inborn (contract support of the University of Minnesota's international registry of malignancies in persons with congenital immune deficiency states) or acquired; i.e., drug-induced in conjunction with renal transplantation (10, 19, 32, 76).

The carcinogenic effects of drugs has been the subject of several review articles during the year (6, 7, 21, 69), and a case-report extends the understanding of liver neoplasia in Fanconi's aplastic anemia after treatment with anabolic androgenic steroids (49).

Our studies of the viral etiology of human cancer have recently concentrated on obtaining specimens from cancer families for special laboratory determinations. Epidemiologic studies pertained to Hodgkin's disease (1, 8, 13, 18) and concerned primarily the discovery of statistical artifacts in the work of other investigators, which erroneously suggested horizontal transmission of the disease.

Host Factors:

A major accomplishment of the Branch during the year was the convening of 90 epidemiologists and other etiologists for a meeting concerning high-risk factors in human carcinogeneses. The meeting was co-sponsored by the Branch, The Division of Cancer Control and Rehabilitation and the American Cancer Society (80). The purpose was to review current understanding of host factors (41, 47), environmental influences and interactions between the two, especially as such understanding relates to improved cancer control. A large number of recommendations were made in this regard, and with respect to future activities concerning studies of etiology and pathogenesis.

Studies of national cancer mortality statistics revealed that, as compared with whites, Chinese in the U.S. have more than a 20-fold excess of nasopharyngeal cancer, and substantial excess of lung, liver and thyroid cancers -- the latter being statistically significant only in males (11). Alaskan natives (Eskimos, Indians and Aleuts) had high mortality rates for cancer of the nasopharynx, esophagus, kidney and salivary glands. Among Alaskan whites only nasopharyngeal cancer was excessive in both sexes, suggesting an environmental determinant (65).

The Branch has continued to lead the nation in studies of familial aggregation of cancer (31, 53, 74). About a dozen families were intensively studied during the year. The most sophisticated paper yet produced in this regard is in press (12). Overviews on the host factors in oncogenesis have sought to integrate information that has developed rapidly in the world literature concerning especially immunology and cytogenetics (9, 36, 41, 43, 47, 52, 73, 77). One such summary took the form of a Venn diagram, that

simplifies the complex relationships between sex chromosomes, gonadal dysgenesis, Turner's syndrome and gonadoblastoma (50).

Comprehensive reviews were also prepared on host and environmental factors in bone cancer (35, 79), multiple primary cancers (25, 62, 73) and leukemia (38, 77).

Children's Cancer:

The Branch has continued to lead the nation in studying children's cancer through clinical epidemiology. During the year a major report, the first of its kind, revealed that long-term survivors of childhood cancer have no increase in the frequency of abnormal progeny (25). Several studies by members of the Branch revealed that Wilms' tumor survival has improved substantially nationwide with increased use of chemotherapy (5, 23, 51, 67). Analysis of data from the End Results Program by members of the Biometry and Epidemiology Branches showed that among children, 5-year survival from 1950-54 through 1964-69 increased only from 29% to 34% (51). Leukemia survival during this interval increased from 1% to 5% (51). Better results can be expected in the future as newer therapy has since been developed.

The first report ever issued in the U.S. of childhood cancer incidence by histologic type was published in conjunction with the Biometry Branch (64). The incidence was substantially higher than the mortality rates even for the earliest most rapidly fatal cancers.

Through the use of death-certificate data for childhood cancer, 1960-69, a report was published on the mortality rates for 16 histologic types according to single year of age, sex and race (46). The mortality patterns differ considerably from one cell type to another, indicating important differences in the origins of the neoplasms. Reports were also published on childhood cancers of specific sites: rhabdomyosarcoma (45), colorectal cancers (3), blood-vessel neoplasms (75) and selected sarcomas (2).

Study of congenital malformations in children with Wilms' tumor (National Wilms' Tumor Survey) confirmed and extended observations previously made by members of the Branch (52).

In conjunction with the Environmental Hazards Committee of the American Academy of Pediatrics (AAP) a sign was prepared for posting in the offices of pediatricians throughout the country. The sign reads, "For the health of our children, please don't smoke." It bears the insignia of the AAP and the National Cancer Program. It will be sent by AAP to 16,000 pediatricians. If each were to post it, and it were seen by an average of 1,000 mothers per pediatrician per year, the message would reach 16 million mothers repeatedly.

Because information on the etiology of childhood cancer is so widely dispersed in the medical literature, the dissemination of important new observations and their significance is often very slow. (It took about 2 years before it was generally known by pediatricians that synthetic estrogen during pregnancy can cause adenocarcinoma of the cervix or vagina in the offspring.) To improve communication of new information and to enhance

interest in etiology, the Branch has issued a monthly Newsletter since December 1973 summarizing and commenting on recently published reports. The Newsletter appears to have been well received and to have achieved its purpose.

Veterinary Medical Data Program (VMDP):

In 1961 the Epizoology Section was initiated in the expectation that research into neoplasia among domestic animals would provide new insight into the origins of human cancer. At that time, there was an absence of systematically collected data for epidemiologic research. The Section developed a procedure for making standard observations on a mass scale to permit machine-processing and retrieval. Fifteen veterinary colleges contribute about 15,000 abstracts per month summarizing diagnostic, demographic and identifying information on each animal discharged. Each contributing institution receives a monthly listing of its data; NCI has the pooled data for its use. Since its beginning, the VMDP has accumulated more than 1 million abstracts, including 25,000 tumors reported during the period March 1964 through December 1973. Because the NCI file has become the largest single source of veterinary clinical data, the Epizoology Section has increasingly functioned as an information source for researchers of diverse interests and nationalities, including those in cancer research centers, the World Health Organization, and colleges of medicine and veterinary medicine. Our own reports issued during the year described the distribution of chemodectoma in dogs, and suggested a possible role of chronic hypoxia in the etiology of that tumor (16); gave the salient features of nervous tissue tumors in domestic animals (17), pancreatic islet cell tumors (59), and pancreatic carcinoma (57) in domestic animals, and estimated their relative frequencies in the hospital population; concluded that occupational exposure to poultry was not associated with a high risk for lymphoma, Hodgkin's disease, leukemia, or all cancers combined (61); and called attention to the first claims of a concommitant geographic variation of esophageal tumors in poultry and man in North China (58). Studies of diseases other than cancer revealed that four breeds of dogs were at excess risk for ectopic ureter, a condition mainly found in bitches, and showing some epidemiologic similarity to ectopic ureter in man (15), and that the patterns of occurrence of congenital umbilical and inguinal hernias were very different among the species studied (14).

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71) Hoover, R. N. and Fraumeni, J. F., Jr.: Industrial bladder cancer in the United States: A geographical evaluation. In Proc. of XI International Cnacer Congress. Amsterdam, Excerpta Medica. In press.

72) Fraumeni, J. F., Jr. and Mulvihill, J. J.: Cancer of specific tissues: At risk of colorectal cancer? In Schottenfeld, D. (Ed.) Cancer Epidemiology and Prevention: Current Concepts. Springfield, Charles C. Thomas, 1975, pp. 404-415.

73) Fraumeni, J. F., Jr.: Multiple primary neoplasms: Relationship to familial cancer. In Severi, L. (Ed.) Multiple Primary Malignant Tumours. Fifth Perugia Quadrennial International Conference on Cancer. In press.

74) Fraumeni, J. F., Jr., Grundy, G. W., Creagan, E. T. and Everson, R. B.: Six families prone to ovarian cancer. Cancer. In press.

75) Chabalko, J. J. and Fraumeni, J. F., Jr.: Blood-vessel neoplasms in children: Epidemiologic aspects. Med. Pediatr. Oncol. In press.

76) Barnes, B. A., Bergan, J. J., Braun, W. E., Fraumeni, J. F., Jr., et al: Twelfth report of the human renal transplant registry. J.A.M.A. In press.

77) Fraumeni, J. F., Jr.: Epidemiology of leukemia. In Proc. of the National Conference on Acute Leukemias. Montreal. In press.

78) Fraumeni, J. F., Jr.: Chemicals in the inducation of respiratory tract tumors. In Proc. of XI International Cancer Congress. Amsterdam, Excerpta Medica. In press.

79) Fraumeni, J. F., Jr.: Bone cancer: Epidemiologic and etiologic considerations. In Vaeth, J. M. (Ed.) Frontiers of Radiation Therapy and Oncology. Vol. X. Basel, S. Karger. In press.

80) Fraumeni, J. F., Jr. (Ed.): Persons at High Risk of Cancer: An Approach to Cancer Etiology and Control. To be published.

81) Blot, W. J., Shimiza, Y., Kato, H., Miller, R. W.: Frequency of marriage and live birth among survivors prenatally exposed to the atomic bomb. Am. J. Epidemiol. In press.

82) Blot, W. J. and Kawamoto, S.: The effects of atomic radiation upon growth and development, J. Radiat. Res. In press.

83) Li, F. P., Melvin, K. E. W., Tashjian, H. A., Jr., Levine, P. H. and Fraumeni, J. F., Jr.: Familial medullary thyroid carcinoma and pheochromocytoma: Epidemiologic investigations. J. Natl. Cancer Inst. 52: 285-287, 1974.

84) Li, F. P., Willard, D. R. and Goodman, R.: Malignant lymphoma after diphenylhydantoin (dilantin) therapy. Cancer. In press.

85) Mulvihill, J. J. (Compiler): Final meeting in the Harriet Lane Home amphitheater. Johns Hopkins Med. J. In press.

ROBERT W. MILLER, M.D.

Chaired Symposium

XI International Cancer Congress, Environmental Causes of Cancer: Epidemiologic Appraisal. Montecatini, Italy, October 21-22, 1974.

Consultant

Children's Hospital of Philadelphia. Advisory Committee, Pediatric Cancer Center Program.
Medical Research Institute, Camden, New Jersey. Advisory Committee for Human Cell Bank.
Advisory Committee for Epidemiology Unit of NCI of Canada.

Committee Memberships

NAS-NRC Advisory Committee on Epidemiology and Veterans Follow-up Studies
NAS-NRC ad hoc Committee to Evaluate the Scientific Program at the Atomic Bomb Casualty Commission
U.S.-Japan Panel on Environmental Mutagenesis and Carcinogenesis
U.S.-Japan Cooperative Cancer Research Program
Chairman, Committee on Environmental Hazards, American Academy of Pediatrics

Lectures

Grand Rounds, Georgetown University Pediatrics Department, July 12, 1974.
Review of Childhood Cancer Epidemiology, Tokyo University, Department of Pediatrics, July 18, 1974.
Alert Practitioner Approach to Cancer Etiology, Atomic Bomb Casualty Commission, Hiroshima, Japan, July 23, 1974.
Overview -- Occupational Cancers, U.S.-Japan Panel on Mutagenesis and Carcinogenesis, Tokyo, Japan, August 5, 1974.
Environmental Causes of Human Cancer, National Cancer Center Research Institute, Tokyo, Japan, August 14, 1974.
Lectured to undergraduate students on Cancer Etiology, UCLA, University of California in Fresno and in La Jolla, September 23-25, 1974.
Viral Etiology of Cancer, Los Angeles Children's Hospital, September 23, 1974.
Epidemiology of Bone Cancer in Children, Gesellschaft zur Bekämpfung der Krebskrankheiten, Dusseldorf, Germany, October 17, 1974.
International Comparison of Childhood Cancer According to Cell Type, XI International Cancer Congress, Florence, Italy, October 24, 1974.
Environmental Cancers, Johns Hopkins School of Public Health, Baltimore, Maryland, November 8, 1974.
Overview -- Host Factors, National Cancer Institute-American Cancer Society Meeting on Persons at High Risk of Cancer: An Approach to Cancer Etiology and Control, Key Biscayne, Florida, December 9, 1974.

International Comparison of Childhood Cancer by Cell Type, U.S.-USSR Epidemiological Working Group, Bethesda, Maryland, February 10, 1975.
National Death Index, Interagency Toxicology Group, Bethesda, Maryland, March 4, 1975.
Bedside Approach to Cancer Etiology, NCI Cancer Advisory Board, Bethesda, Maryland, March 17, 1975.
Etiology of Childhood Cancer, Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania, March 19, 1975.
Grand Rounds: Environmental Causes of Childhood Cancer, Montefiore Hospital Department of Pediatrics, New York City, New York, March 20, 1975.
Recent Developments in Etiology of Childhood Cancer, St. Jude Hospital, Memphis, Tennessee, March 21, 1975.
Features in Common of Persons at High Risk of Leukemia, Biology of Radiation Carcinogenesis, Gatlinburg, Tennessee, April 7, 1975.
Epidemiology of Childhood Cancer, Birth Defects Conference, Kansas City, Missouri, June 2, 1975.
Epidemiology of Mental Retardation, Rachford Lectureship, Cincinnati, Ohio, June 12, 1975.

Associate Editor

Journal of the National Cancer Institute
Teratology

JOSEPH F. FRAUMENI, JR., M.D.

Committee Memberships

Chairman, NIH Committee on Transplantation Research
Cause and Prevention Executive Staff, National Cancer Institute
Field Studies and Statistics Program Review Group, National Cancer Institute
Scientific Advisory Committee, Cancer Control Bureau, New York State Department of Health
Epidemiology Committee, Breast Cancer Task Force, National Cancer Institute
Advisory Committee, American College of Surgeons Renal Transplant Registry
Editorial Advisory Board, Cancer Research
Editor, Medical and Pediatric Oncology
Journal Policy Committee, Teratology Society
Smoking and Health Review Board, National Cancer Institute
Committee on Cancer Prevention and Detection, American Society of Clinical Oncology
Advisory Committee, Cancer Epidemiology Unit, University of Southern California School of Medicine
U.S. Delegation, U.S.-USSR Joint Working Group on Cancer Epidemiology

Conferences

Panel Chairman -- Genetic Factors. XI International Cancer Congress, Florence, Italy, October 23, 1974.

Conference Chairman -- Persons at High Risk of Cancer: An Approach to Cancer Etiology and Control. National Cancer Institute and American Cancer Society, Key Biscayne, Florida, December 10-12, 1974.
Symposium Chairman -- On the Curability of Cancer. Society for Epidemiologic Research, 8th Annual Meeting, Albany, New York, June 20, 1975.

Lectures

"Bone cancer: Epidemiologic and etiologic considerations." Tenth Annual San Francisco Cancer Symposium, San Francisco, California, September 27, 1974.
"Immuno-epidemiology." National Cancer Advisory Board Meeting, Bethesda, Maryland, October 7, 1974.
"Chemicals in the induction of respiratory-tract tumors." XI International Cancer Congress, Florence, Italy, October 22, 1974.
"Congenital malformations and cancer." BIOL 523 -- Malformation in Developmental Biology. NIH Graduate Program -- Foundation for Advanced Education in the Sciences, Inc., Bethesda, Maryland, January 7, 1975.
"Genetic and immunologic determinants of cancer." Department of Microbiology, Howard University School of Medicine, Washington, D.C., January 31, 1975.
"Familial susceptibility to cancer." Joint Working Group for Cancer Epidemiology, U.S.-USSR Cancer Program, Bethesda, Maryland, February 10, 1975.
"Genetic factors in cancer etiology." Clinical Center, National Institutes of Health, Bethesda, Maryland, February 19, 1975.
"Persons at High-Risk of Cancer: Summary of Workshop." National Cancer Advisory Board Meeting, Bethesda, Maryland, March 17, 1975.
"Epidemiology of Pancreatic Cancer." Howard University School of Medicine, Washington, D.C., March 18, 1975.
"Discussion summary: Inorganic metals and cancer." Conference on Occupational Cancer, New York Academy of Sciences, New York, New York, March 17, 1975.
"Familial aggregation of cancer." Conference on Cancer Detection and Prevention, American Cancer Society, Denver, Colorado, May 2, 1975.
"Epidemiology of cancer of the biliary tract and pancreas." Conference on Nutrition and Cancer, American Cancer Society, Key Biscayne, Florida, May 21, 1975.

WILLIAM A. PRIESTER, DVM

Conferences

International Symposium on Animal Disease Monitoring, Guelph, Ontario, Canada, July 4-5, 1974 -- Participant.
Persons at High Risk of Cancer: An Approach to Cancer Etiology and Control, Key Biscayne, Florida, December 10-12, 1974 -- Rapporteur.

Lectures

"Genetic determinants of cancer in man and animal," Combined Medical-Veterinary Staff Conference, University of California, Davis, October 10, 1974.
"Studies on spontaneous animal tumors for clues to cancer in man," Environmental Carcinogenesis Collaborative Group, Bethesda, Maryland, March 4, 1975.

JOHN J. MULVIHILL, M.D.

Committee Memberships

American Academy of Pediatrics, Committee on Environmental Hazards
Journal of the National Cancer Institute, Board of Editors

Lectures

"Prenatal sex-hormone exposure and cardiac defects in man," 14th Annual Teratology Society Meeting, Vancouver, British Columbia, July 9, 1974.
"Osteosarcoma, skeletal anomalies, and erythrocytic macrocytosis in an American Indian family," 26th Annual Meeting, American Society of Human Genetics, October 18, 1974.
"Genetic and congenital defects," Workshop on Persons at High Risk of Cancer: An Approach to Cancer Etiology and Control, Key Biscayne, Florida, December 10, 1974.
"Genetic determinants of cancer," Second Meeting of the U.S.-USSR Joint Working Group for the Study of the Epidemiology of Cancer, Bethesda, Maryland, February 10, 1975.
"Genetic factors in pulmonary neoplasia," 1975 Birth Defects Conference, Kansas City, Missouri, June 2-4, 1975.

ROBERT N. HOOVER, M.D.

Committee Memberships

NIH Committee on Transplantation Research

Conferences

Society for Epidemiologic Research, Annual Meeting, Berkeley, California, 1974.
XI International Cancer Congress, Florence, Italy, October 25, 1974.

Lectures

"Risk of cancer in renal transplant recipients," Seminar at Johns Hopkin's University, February 7, 1975.
"Cancer by county: New resource for etiologic clues," U.S.-USSR Joint Working Group on Epidemiology, February 10, 1975. Also presented at Multiagency Committee on Carcinogenesis, March 4, 1975.
"Industrial bladder cancer in the United States: A geographical evaluation, XI International Cancer Congress, Florence, Italy, October 25, 1974.

FREDERICK P. LI, M.D.

Consultant

Biometry and Epidemiology Contract Review Committee, National Cancer Institute
Late Effects Study Group, National Cancer Institute

Presentations

Risk of Second Neoplasms in Survivors of Childhood Cancer, Plenary sessions, American Society of Clinical Oncology, Houston, Texas, April, 1974.

WILLIAM J. BLOT, PH.D.

Lectures

"Cell mediated immune response in a rural preschool population," American Public Health Association, 102nd Annual Meeting, New Orleans, Louisiana, October 23, 1974.
"Interactions of growth and morbidity among preschool children in rural India," American Public Health Association, 102nd Annual Meeting, New Orleans, Louisiana, October 24, 1974.

Conferences

Persons at High Risk of Cancer: An Approach to Cancer Etiology and Control, Key Biscayne, Florida, December 10-12, 1974 -- Rapporteur.

HOWARD M. HAYES, D.V.M.

Lectures

"Future of the Veterinary Medical Data Program," American Association of Veterinary Medical Records Administrators, American Veterinary Medical Association Convention, Denver, Colorado, July 23, 1974.
"Clinical Applications of the Veterinary Medical Data Program," Auburn University, School of Veterinary Medicine, September 24, 1974.
"A National Disease Reporting System," Colloquium on Advances in Veterinary Medicine 1, Louisiana State University, School of Veterinary Medicine, September 27, 1974.
"U.S. Cancer Mortality by County, 1950-69: It's Utility in Epidemiologic Investigations," National Cancer Institute's Administrative Officers Executive Staff Meeting, Building 31, NIH, Bethesda, Maryland, December 10, 1974.
"Veterinary Cancer Epidemiology," Academy of Health Sciences, Health Services Command, U.S. Army, Fort Sam Houston, Texas, February 12, 1975.

Serial No. ZO1-CP-04325-13-E
1. Epidemiology Branch, OADFS&S, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Planning and Development in Cancer Epidemiology

Previous Serial Number: NCI-4325

Principal Investigator: Robert W. Miller, M.D.

Other Investigators: J. F. Fraumeni, Jr., M.D., W. A. Priester, DVM, and other professional staff of the Epidemiology Branch

Cooperating Units: None

Man Years:

Total	2.0
Professional	2.0
Other	0.0

Project Description

Objectives:

1. To originate new epidemiologic approaches to the study of cancer causation in man and animals.
2. To develop sources of data related to specific epidemiologic problems.
3. To stimulate epidemiologic research in other health agencies.
4. To develop on-the-job training for medical and statistical officers.

Methods Employed:

The Program is based on:

1. Leads from animal experimentation, laboratory research and clinical observations.
2. Prospective studies (in retrospect) which relate cancer occurrence to events recorded prior to the onset of cancer in medical examinations obtained in a standard fashion from large numbers of persons (examples -- clinical health-surveys, and military medical examinations).

3. Retrospective studies based on questionnaires obtained by personal interview or by mail, for a comparison of persons (examples -- clinical health-surveys, and military medical examinations).

4. "Laterospective" studies which concern the detection from clinic records of the excessive concurrence of cancer with pre-existent disease, such as congenital defects or autoimmune disorders.

As specific epidemiologic questions arise from laboratory or clinical observations, sources of field data are developed to answer these. Conversely, the Branch seeks by its surveys to raise questions which can be answered by laboratory or clinical studies. Parallel with this, to fill the tremendous deficiency in qualified personnel, the selection and training of young physicians, veterinarians, and statisticians must take place. The principles they learn from the guided planning, execution, and analysis of a program in cancer epidemiology are applicable to studies of the genesis of congenital malformations, heart disease, arthritis, mental aberrations, or the effects of ionizing radiation.

Significance to Bio-medical Research and the Program of the Institute:

If the example set by the Branch is a good one, interest will be stimulated among medical scientists in the use of epidemiologic methods for their research. In consequence, there may develop a further recognition of the usefulness of office and hospital records for survey studies. With this would go an appreciation for the need to adapt the standard medical records for epidemiologic research. These developments, in turn, could promote an interest in looking beyond the walls of hospitals and medical centers for opportunities in medical research.

Proposed Course of Project:

To develop opportunities in cancer epidemiology as they arise. During the past year an "Alert Practitioner Program" was initiated at the Boston Field Station in conjunction with similar NIEHS-sponsored feasibility studies in the Pediatrics Departments at Montefiore Hospital (New York City), Duke University and Los Angeles County-USC Medical Center. Under faculty supervision, medical students obtain special etiological histories and pedigrees from parents whose children have cancer or other diseases of unknown etiology. The purpose is to increase attention to etiology in medical schools, to seek new clues to causes of disease and to identify students with an aptitude to think epidemiologically, who can then be advised as to further training in this regard.

Serial No. ZO1-CP-04371-11-E
1. Epidemiology Branch, OADFS&S, DCCP
2. Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: National Cancer Institute Veterinary Medical Data Program

Previous Serial Number: NCI-4371

Principal Investigator: W. A. Priester, DVM

Others Investigators: H. M. Hayes, DVM

Cooperating Units: School and Colleges of Veterinary Medicine: Michigan State University, University of Missouri, University of Minnesota, Iowa State University, Ontario Veterinary College, Purdue University, University of Georgia, Kansas State University, University of Illinois, University of California, Ohio State University, University of Saskatchewan, Colorado State University, Auburn University.

Also, see Project Report NCI-4380-5.

Man Years:

Total	:	2.40
Professional	:	.40
Other	:	2.00

Project Description

Objectives:

To provide animal disease data as a research resource. To coordinate the program with related programs primarily in area of disease nomenclature, terminology and code. To develop ways and means of data utilization applicable to comparative medical studies.

Methods Employed:

Standard data processing methods. Details are described in related Project Reports and Contract Narratives of Epizoology Section.

Major Findings:

Not applicable as this is primarily a service activity. The National Cancer Institute Veterinary Medical Data Program is currently processing data from 12 veterinary colleges in this country and 2 in Canada. The program has been endorsed and implemented in Europe under the auspices of WHO. For the first time, veterinary medicine and the medical sciences have benefit of a national and international animal disease data system. A number of cooperative studies were begun during the year with faculty members at the various participating colleges of veterinary medicine.

Significance to Bio-medical Research and the Program of the Institute:

The comparative (veterinary) medical data retrieval system instituted at the College of Veterinary Medicine, Michigan State University on a pilot basis (PH 43-64-85) was undertaken to design a system for collection, storage and retrieval of veterinary medical clinical data. The program is now fully operative and has been extended to 13 additional veterinary colleges. Establishment and maintenance of reliable sources of veterinary medical data provide a research resource, hitherto unavailable, that is applicable not only to cancer research but to research areas such as infectious diseases, congenital defects, and gerontological diseases. Continued NCI Epidemiology Branch animal studies research function depends upon continued availability of valid domestic animal disease data, particularly as regards neoplastic disease. The data bank, which included information on more than 1,000,000 patients through 1973, is often used as a reference source by scientists at NCI and elsewhere.

Proposed Course of Project:

Additional veterinary schools will be added to the program as interest and capability of the individual applicants are established. It is anticipated that this project will continue for a minimum of two years.

Date initiated: October 22, 1965

Current annual level: $34,000

Publications

Priester, W.A.: Collecting and using veterinary clinical data. In: Proceedings of International Symposium on Animal Disease Monitoring. Chas. C. Thomas, Springfield. In press.

Serial No. Z01-CP-04377-05-E
1. Epidemiology Branch, OADFS&S, DCCP
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Familial and Genetic Factors in Malignancies

Previous Serial Number: NCI-4377

Principal Investigators: Joseph F. Fraumeni, Jr., M.D., Robert W. Miller, M.D. and John J. Mulvihill, M.D.

Other Investigators: Frederick P. Li, M.D., William A. Blattner, M.D., Thomas W. Pendergrass, M.D. and Richard B. Everson, M.D.

Cooperating Units: Various NCI clinical and experimental groups

Man Years:

Total	:	3.25
Professional	:	2.25
Other	:	1.00

Project Description

Objectives:

To document the occurrence of patterns of family aggregation of neoplasms. To study such families by genetic and laboratory investigations, in an effort to elucidate the degree to which heredity and/or the common familial environment contribute to the etiology of neoplasms. To distribute tissue and blood specimens from such families to interested laboratory investigators, for etiologic study by cytogenetic, immunological, viral, and tissue culture methods. To identify genetic factors and disorders associated with cancer.

Methods Employed:

Interview of cancer patients with respect to family occurrence of cancer and other disorders, as well as information on prior medical and environmental history; documentation of history by obtaining appropriate vital records and hospital charts; collection and distribution of biological specimens from such families; review of hospital records of series of patients with selected genetic diseases or neoplasms; survey of childhood cancer death certificates in the U.S. since 1960 to identify sib aggregation.

Major Findings:

A number of families have been studied with cancers of single sites and of multiple sites. Many of the tumors which occur together in familial syndromes also coexist excessively in studies of multiple primary cancer, suggesting that a familial cancer syndrome may represent a "scattering" over the family tree of tumors which share etiologic influences. The results of laboratory studies of specimens obtained from members of various cancer families are being assembled.

Reports were published on 1) the risks of childhood tumor patients for a second primary neoplasm and for cancer in first degree relatives (parent, sib or child); 2) individual families with colonic cancer and lymphoproliferative disorders; and 3) high titers of antibody to the Epstein-Barr virus in relatives of cancer patients. Didactic notes appeared on the relation of the Y chromosome to gonadal malignancy and on the merits of studying parents for etiologic clues in childhood cancer. Statistical pitfalls in studying familial aggregations were discussed and two reviews of genetic factors in human cancer were prepared. Studies in progress concern collaborative etiologic studies on leukemic patients being tested for the candidate virus of Gallagher and Gallo. Also under study are familial aggregations of male breast cancer, osteosarcoma, acute leukemia, and other lymphoproliferative disorders.

Significance to Bio-medical Research and the Program of the Institute:

Epidemiologic surveys and detailed studies of families at high risk of cancer may help to detect environmental and genetic influences in carcinogenesis. In addition, identification of these families has therapeutic implications, enabling surveillance and early diagnosis of neoplasms.

Proposed Course of Project:

The same approach will be continued. New laboratory methods and epidemiologic clues from other sources will be incorporated into the project protocol as necessary.

Publications

Mulvihill, J.J.: Cancer in congenital and genetic disease. In Persons at High Risk of Cancer: An Approach to Cancer Etiology and Control. In press.

Fraumeni, J.F., Jr., Wertelecki, W., Blattner, W.A., Jensen, R.D., Leventhal, B.G.: Varied manifestations of a familial lymphoproliferative disorder. Am. J. Med. In press.

Miller, M., Costanza, M., Li, F.P., et al: Familial colon cancer. Cancer. In press.

Levine, P.H., Fraumeni, J.F., Jr., Reisher, J.I., and Waggoner, D.E.: Antibodies to Epstein-Barr virus-associated antigens in relatives of cancer patients. J. Natl. Cancer Inst. 52: 1037-1040, 1974.

Li, F. P. and Jaffe, N.: Progeny of childhood-cancer survivors. Lancet 2: 707-714, 1974.

Li, F.P., Cassady, J.R. and Jaffe, N.: Risk of second tumors in survivors of childhood cancer. Cancer. In press.

Mulvihill, J.J., Ridolfi, R.L., Schultz, F.R., Borzy, M.S. and Haughton, P.B.T.: Hepatic adenoma in Fanconi anemia treated with oxymetholone. J. Pediatr. In press.

Mulvihill, J.J., Wade, W.M. and Miller, R.W.: Gonadoblastoma in dysgenetic gonads with a Y chromosome. Lancet. In press.

Blot, W.J.: Familial Hodgkin's disease. Lancet 2: 1520, 1974.

Serial No. ZO1-CP-04378-03-E
1. Epidemiology Branch, OADFS&S, DCCP
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: U.S. Cancer Mortality Survey

Previous Serial Number: NCI-4378

Principal Investigator: Thomas J. Mason, Ph.D.

Other Investigators: Joseph F. Fraumeni, Jr., M.D., Frank W. McKay, Jr., Robert N. Hoover, M.D., and William J. Blot, Ph.D.

Cooperating Units: National Center for Health Statistics

Man Years:

Total	4.75
Professional	3.25
Other	1.50

Project Description

Objectives:

To examine the cancer mortality experience in the United States relative to cancer etiology for specific races, White, Black, American Indian, Chinese, Japanese, other, and also for individual counties within states.

Methods Employed:

Computer analysis of over 6 million death certificates by site, sex, race, state, and age. The investigation is ongoing, updated year by year, and expanding. Base populations for Whites, Blacks, and all nonwhites are maintained for the entire time period. The system for the total U.S. provides race, sex, ICD, and age-specific analyses of cancer mortality consisting of numbers dead, populations at risk, age-specific and age-adjusted rates, a measure of the variability in the age-adjusted rate, and a linear regression of the age-adjusted rates as a function of time. This system is fully operational.

A system for the analysis of cancer mortality for individual counties with the flexibilities of the national system is currently operational.

Major Findings:

We have completed the first systematic presentation of the geographic distribution of cancer mortality in the United States at the county level. This atlas presents computer-generated maps by site for the time period 1950-1969, and is a companion volume to our earlier publication of cancer mortality rates for individual counties in the United States. These maps should serve to identify counties, or clusters of counties, with elevated cancer rates which in turn may provide etiologic clues. We detected excesses for cancers of the stomach, bladder, and melanoma of the skin which were consistent with etiologic hypotheses concerning ethnicity (stomach), chemical exposures (bladder), and sunlight (melanoma). This publication should facilitate control programs in high-risk communities for early detection and prevention of cancer.

Counties in the United States were identified with chemical establishments whose primary manufacturing processes use vinyl chloride. Site-specific cancer mortality comparisons revealed an excess of multiple myeloma in males associated with two of the manufacturing categories, synthetic rubber and synthetic fibers. A causal relationship between these manufacturing categories and multiple myeloma could not be established. However, excessive non-Hodgkin's lymphomas have been reported among workers occupationally exposed to vinyl chloride. An industry-based assessment of the occupational contribution to the excess of multiple myeloma is needed to evaluate the etiologic importance of this relationship.

Geographic analysis of U.S. cancer mortality, 1950-69, revealed excess rates for bladder, lung, liver and certain other cancers among males in 139 counties where the chemical industry is most highly concentrated. The correlation could not be explained by confounding variables such as urbanization, socioeconomic class, or employment in non-chemical industries. If the excess cancer mortality in these areas is due to industrial exposures, the actual risk of cancer among certain chemical workers must be very high. The correlation was limited to counties associated with specific categories of the chemical industry; many involve known occupational hazards, while others suggest leads to chemically-induced cancer in man.

Because exposures to man-made ionizing radiation have induced leukemia and other cancers, it is frequently asked whether human cancer rates are higher in mountainous areas, because of cosmic radiation. Data from U.S. death certificates, 1950-69, revealed no excess mortality from leukemia or other cancer in counties with most of their land mass at 3000+ feet elevation or in Denver County or Salt Lake County.

The possible relationship between human cancer deaths and exposure to poultry was studied in 1019 U.S. counties for 1950-69. Deaths from uterine cervical and ovarian cancers and from multiple myeloma were excessive in high poultry population areas; the significance for the latter two cancers disappeared when data from the high poultry population areas were compared with data from the total United States. The excess of uterine cervical cancer was probably due

to previously defined social determinants rather than to exposure to poultry. The study did not detect any excess risk of lymphomas, Hodgkin's disease, and leukemia in high poultry population areas.

Significance to Bio-medical Research and the Program of the Institute:

This survey provides a continually expanding data set against which specific etiological hypotheses about cancer can be tested. The capability of subdivision of the data set into specific racial and geographical subsets (specifically county level analysis) provides an opportunity to more critically test hypotheses regarding the etiology of cancer than was possible at an earlier point in time.

Proposed Course of Project:

Areas in the United States with elevated cancer mortality rates will be characterized with regard to manufacturing in an attempt to assess the relative contribution of occupation. The effects of urbanization, socioeconomic status and ethnic composition of the population will also be investigated for these areas with elevated mortality. The contiguous United States has been subdivided into subunits as a function of latitude, urbanization, and socioeconomic status independent of mortality. An assessment of the effect of these variables, individually and collectively, is ongoing.

Methods for the systematic evaluation and presentation of the geographic distribution of cancer mortality among nonwhites are being developed and evaluated.

For anatomic sites whose distributions of age-specific rates are bi-modal, an investigation of characteristic differences for geographic subunits is ongoing.

Additional sources of demographic data for counties in the U.S. are being sought.

Publications

Mason, T.J., McKay, F.W., Hoover, R., Blot, W.J., Fraumeni, J.F., Jr.: Atlas of Cancer Mortality for U.S. Counties: 1950-1969, DHEW Publication No. (NIH) 75-780. In press.

Mason, T.J.: Cancer mortality in U.S. counties with plastics and related industries. Environ. Health Perspect. In press.

Hoover, R.N., Fraumeni, J.F., Jr.: Cancer mortality in U.S. counties with chemical industries. Environ. Res. In press.

Mason, T.J., Miller, R.W.: Cosmic radiation at high altitudes and U.S. cancer mortality, 1950-1969. Radiat. Res. 60: 302-306, 1974.

Priester, W.A., Mason, T.J.: Human cancer mortality in relation to poultry population, by county, in 10 Southeastern states. J. Natl. Cancer Inst. 53: 45-49, 1974.

Serial No. ZO1-CP-04379-07-E
1. Epidemiology Branch, OADFS&S, DCCP
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of Congenital Defects in Domestic Animals

Previous Serial Number: NCI-4379

Principal Investigator: Howard M. Hayes, D.V.M.

Other Investigators: Thomas W. Pendergrass, M.D.

Cooperating Units: See Project Report NCI-4371-11

Man Years:

Total	.40
Professional:	.20
Other	.20

Project Description

Objectives:

To identify animal models useful in congenital and cancer research in humans.

Methods Employed:

From case summaries submitted to the Veterinary Medical Data Program (NCI-4371), series of animals with congenital defects are systematically analyzed in relation to the total number of animals seen in the same time period. A general survey of the findings has already been published for cases seen from March 1964 through January 1969. Specific studies include congenital umbilical and inguinal hernias in domestic animals, congenital ocular defects, and reports about dogs with cardiovascular defects, patellar dislocation and hip dysplasia. During this year a study has appeared about ectopic ureter in dogs. Currently in progress is a study about cryptorchidism in dogs.

Major Findings:

Four canine breeds were identified at high risk for ectopic ureter (Siberian Husky, West Highland White Terrier, Fox Terrier, Miniature and Toy Poodle). Several epidemiologic features of this anomaly in dogs were similar to those in humans.

Significance to Bio-medical Research and the Program of the Institute:

Experiments on animals are essential to progress in medical research. Traditionally, a small number of species is used for laboratory experimentation. Currently popular laboratory animals are generally poor models for genetic disease and congenital malformations in man, and the rarity of these individual conditions in the human population limits clinical research. Our project attempts to detect conditions spontaneously occurring in domestic animals which are more nearly homologous to human afflictions. Taking heart malformations in dogs as an example, we have defined high frequencies of 1) patent ductus arteriosus in Miniature/Toy Poodles, and 2) persistence of the right aortic arch in German Shepherds. Since certain congenital malformations in man are associated with childhood malignancies, we hope to identify domestic animals which may serve as research models to explore the borderland between teratology and oncology.

Proposed Course of Project:

Data will continue to come from the Veterinary Medical Data Program and will be analyzed for breed-specific lesions.

Publications

Hayes, H.M.: Ectopic ureter in dogs: epidemiologic features. Teratology 10: 129-132, 1974.

Serial No. ZO1-CP-04380-05-E

1. Epidemiology Branch, DADFS&S, DCCP
2. Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Study of the zoographic characteristics of domestic animals with tumors.

Previous Serial Number: NCI-4380

Principal Investigator: W. A. Priester, DVM

Other Investigators: Howard M. Hayes, DVM

Man Years:

Total	:	1.40
Professional	:	.40
Other	:	1.00

Project Description

Objectives:

To provide a reference of the distribution of spontaneous tumors in domestic animals. To search for unusual distribution of tumors, within a large, animal hospital population, which might suggest possible clues regarding the etiology of cancer.

Methods Employed:

Using patients with tumors reported to the Veterinary Medical Data Program as case series, and all patients seen during like periods as population references, the following will be studied:

Characteristics of patients: age, breed/species, sex
Characteristics of tumors: degree of malignancy, site, and histogenic type.

In addition, other factors may be studied as the analyses progress.

Major Findings:

25,000 tumors were reported during the period March 1964 through December 1973 among 500,000 animal-years-at-risk. The proportion of patients with tumors was highest among dogs and lowest among horses, when the four major species

categories, bovine, equine, feline, and canine, were considered. In almost all of the studies of specific tumors conducted so far age has been generally associated with an increasing risk for tumors but not to the same degree for each specific tumor type. Invariably, one or more breeds of dogs, can be identified as being at excess risk for the specific tumor type under study. While no striking associations between specific tumor type and other primary tumors in the same patient have been noted, several associations were identified that seemed similar to conditions described for man.

Significance to Bio-medical Research and the Program of the Institute:

Animal studies done by the Branch have demonstrated several relationships of interest in cancer research -- bone sarcoma and size, canine lymphoma and certain breeds, canine mastocytoma and breed ancestry. This study is designed to detect high risk groups in a systematic fashion. The results may well define animal subgroups suitable for studies of cancer etiology that would be impractical in a human population.

Proposed Course of Study:

Data will be added to the tumor file and reference population base at the end of each fiscal year. While some high-frequency tumors will be studied, the main effort will be directed towards study of those spontaneous tumors in domestic animals that have been so infrequently reported in the literature that characterization of patients with these tumors has never been done.

Date initiated: May 1970.

Current annual level: $19,000.

Publications

Priester, W.A.: Data from eleven United States and Canadian colleges of veterinary medicine on pancreatic carcinoma in Domestic Animals. Cancer Res. 34: 1372-1375, 1974.

Hayes, H.M., Jr., Priester, W.A. and Pendergrass, T.W.: Occurrence of nervous tissue tumors in cattle, horses, cats, and dogs. Int. J. Cancer 15: 39-47, 1975.

Priester, W.A.: Pancreatic islet cell tumors in domestic animals. Data from 11 colleges of veterinary medicine in the United States and Canada. J. Natl. Cancer Inst. 53: 227-229, 1974.

Serial No. 701-CP-04400-15-E
1. Epidemiology Branch, OADPS&S, DCCP
2. Boston Field Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Epidemiology Branch Field Station, Boston, Massachusetts

Previous Serial Number: NCI-4400

Principal Investigator: Frederick P. Li, M.D.

Other Investigators: None

Man Years:

Total	:	1.8
Professional	:	1.0
Other	:	0.8

Project Description

Objectives:

1) To study the epidemiology of human neoplasms and to develop etiologic hypotheses through close observation of individual patients at the bedside.

2) To study the physiologic, psychologic, oncogenic and genetic sequelae of successful cancer therapy in man.

3) To provide consultation in cancer epidemiology to staff physicians and trainees in the Boston area, and to stimulate therapists to observe and report clinical findings of etiologic significance. Also, to undertake collaborative studies with other epidemiologists at the Harvard School of Public Health.

Methods Employed:

Nearly 300 questionnaire interviews of childhood cancer patients have been completed; the breadth of the questionnaire was increased this year. Laboratory studies have been carried out in several families with strong histories of cancer to identify possible inherited defects or environmental risk factors. In addition, a registry has been started of long-term survivors of childhood cancer. These patients and their progeny are being followed for development of new tumors and for evidence of later complications of cancer therapy. Also, childhood cancers in sibs and other close relatives are being systematically recorded for analysis. Attempts are now under way to enlist

other cancer centers to participate in the registries of late effects, multiple primary cancers, and sib-aggregates.

Major Findings:

1) Thirty-seven sibships with childhood cancer have been collected, revealing a high frequency of cancer at a relatively early age in parents and other close relatives.

2) A 20-fold higher risk of a new cancer was found in childhood cancer survivors as compared with persons in the general population.

3) Performance status among 142 survivors of childhood cancer was found to be satisfactory in nearly all patients.

4) Occult colon cancer was detected in a member of a high-risk family through assay for tumor markers.

5) Several families with multiple cases of hematologic cancers are under study. In members of one family, preliminary data suggest defective cellular repair of DNA breaks following radiation injury.

Significance to Bio-medical Research and the Program at the Institute:

Studies at the Field Station generate etiologic hypotheses for clinical and laboratory testing. The studies also help evaluate the relevance for human populations of etiologic data provided by other investigators at the Institute. The teaching of cancer epidemiology provided to medical students and junior house staff may help to stimulate interest in cancer epidemiology and to influence the selection of a career in this field.

Proposed Course of Project:

The Field Station will continue to focus on studies of the epidemiology of childhood cancers. The emphasis will be on detection of clues to etiology through careful clinical study of the natural history of cancer in individual patients. The new questionnaire studies will hopefully provide clues to cancer etiology which were not evident from retrospective reviews of prior data sources. The registry of long-term survivors will provide data on the inheritance of childhood cancers, and on oncogenesis following cancer therapy.

Serial No. Z01-CP-04401-02-E
1. Epidemiology Branch, OADFS&S, DCCP
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunologic Factors in Cancer Etiology

Previous Serial Number: NCI-4401

Principal Investigator: Robert N. Hoover, M.D.

Other Investigators: Joseph F. Fraumeni, Jr., M.D.

Man Years:

Total	:	1.5
Professional	:	0.5
Other	:	1.0

Project Description

Objectives:

1) To identify and study populations with altered immunologic states experiencing unusual rates of malignancy.

2) To study these populations in order to identify the characteristics and possible determinants of any unusual risk.

Studies and Methods Employed:

1) An ongoing follow-up study of the malignancy experiences of renal transplant recipients was continued and updated. In the past year 9785 patients transplanted at 232 hospitals and reported to a renal transplant registry were followed up for incident cases of cancer. Cancer incidence rates from the general population were applied to the age, sex, and time-specific years at risk accumulated by the transplant recipients and various subgroups of the population, in order to develop expected numbers of cancer to compare to this numbers actually observed.

2. Data were collected for a case-control study of cancer in the transplant population described above. For each cancer case, two controls were selected, matched for hospital and year of transplant. Tissue-typing information for cases and controls was requested from all of the hospitals.

3. In collaboration with the University of Minnesota, through the contract mechanism, a Registry of malignancy in patients with immune deficiency syndromes was maintained. Using this Registry as a case identifying mechanism for a case-control study of children with cancer, data are currently being collected from the hospital records of immune-deficient children who developed cancer and those that did not. The data collected concentrate on laboratory tests of immune status and family history of malignancy.

4. A follow-up study is underway of the malignancy, mortality and reproductive experience of 5,000 former employees of Fort Detrick (formerly a biologic warfare research center). A large proportion of these workers fall into a group which would be considered "hyperimmunized." Thus far the personal and immunization records for this population have been abstracted and computerized. Currently a mailing is being prepared to determine the vital status of the members of this group and the health histories of those still living.

5. A reanalysis of a Chicago based study concerning neonatal BCG vaccination and the risk of leukemia and other cancers was conducted by matching a roster of vaccinees with the Childhood Cancer Registry.

Major Findings:

1. The risk of lymphoma among transplant recipients is about 25-times greater than expected, derived almost entirely from a risk of reticulum cell sarcoma that was 350-times greater than expected.

2. The excess risk of lymphoma appeared within a year of transplant and showed a highly unusual predilection for localization to the brain.

3. Other cancers were twice as common as expected, due largely to soft-tissue sarcomas, hepatobiliary carcinomas, lower urinary tract carcinomas and adenocarcinomas of the lung.

4. Age at transplantation is related to the risk of the non-lymphomatous tumors, being higher among the young. On the other hand, the risk of lymphoma may be related to the interaction of the recipient's and donor's ages.

5. There is a remarkable lack of variability of the risk according to a number of recipient and donor characteristics.

6. Persons with immune-deficiency syndromes have a high risk of lymphoma, similar to that among transplant recipients. Unlike the transplant population, however, they also appear to be at high risk of Hodgkin's disease, lymphatic leukemia (children), and stomach cancer (adults).

7. Upon reanalysis, and with the ability to calculate a reasonable denominator, the previously reported protection of 6-fold for leukemia and 4-fold for all cancers for children vaccinated neonatally with BCG were found to be in error. The "protection" was found to be on the order of 2-fold for both of these

categories. Since the methodology of these studies only allows biases in the direction of protection to operate, it was felt that this weak association could not be interpreted as support for the hypothesis of biologic protection against cancer associated with this non-specific vaccination.

Proposed Course of Project:

1. The same population of transplant recipients is being followed and will be re-evaluated yearly.

2. The collaboration with the immunodeficiency and cancer registry will continue with periodic evaluation of the data collected.

3. In the coming year data from the case-control studies described above will be analyzed.

4. The follow-up and analysis of the "hyperimmunized" group described above will be continued.

5. A number of studies relevant to this topic in collaboration with the Veteran's Administration have been designed and are currently being considered for implementation by the V.A. It is hoped that retrospective cohort studies of patients with various immune-deficiency syndromes and others undergoing procedures and drug therapies involving immunosuppression can be initiated in the near future.

Publications

Hoover, R.: Risk of cancer in renal transplant recipients. Am. J. Epidemiol. 100: 526, 1974.

Spector, B.D., Good, R.A., and Kersey, J.H.: Cancer in adults with primary immunodeficiency diseases. Cancer, 1975. In press.

Kersey, J.H. and Spector, B.D.: Immunodeficiencies and malignancies. In Persons at High Risk of Cancer: An Approach to Cancer Etiology and Control. In press.

CONTRACT NARRATIVE
(Summary of Personal Service Contracts)
Fiscal Year 1975
Animal Studies

VETERINARY MEDICAL DATA PROGRAM

Personal Service Contracts between the Epizoology Section and Colleges of Veterinary Medicine at the University of Missouri, Michigan State University, Iowa State University, Ontario Veterinary College, and the University of Georgia, University of Illinois, Kansas State University, Ohio State University, University of Saskatchewan, Purdue University, University of Minnesota, University of California, Colorado State University, and Auburn University.

Title: Purchase Veterinary Case Data.

Objectives: To provide reliable data on disease in domestic animals to Epizoology Section and to further encourage adoption of standard nomenclature and coding of animal disease data. To make available to veterinary medicine, comparative medical research and the scientific community the developments of the Veterinary Medical Data Program.

Methods Employed: Purchase of completed veterinary medical case abstracts in terms of National Cancer Institute Veterinary Medical Data Program (as detailed in related Contract Narratives).

Major Findings: None, as this is primarily a service activity.

Significance to NCI Program and Bio-medical Research: Increase store of data in the Veterinary Medical Data Program. Of particular importance in terms of study of low prevalence diseases (cancer, etc.), geographical distribution and absolute numbers.

Proposed Course of Project: Minimum of two years. Emphasis will be directed towards shifting more responsibility for quality control and for preparation of machine-readable data to the participants.

Dates Initiated:

Michigan State University	-	November 1965
Iowa State University	-	October 1965
Ontario Veterinary College	-	July 1966
University of Georgia	-	January 1967
University of Missouri	-	February 1967
University of Minnesota	-	December 1967
University of Illinois	-	January 1968
Kansas State University	-	January 1968
Purdue University	-	January 1968
Ohio State University	-	January 1969
University of Saskatchewan	-	January 1969
University of California	-	July 1970
Colorado State University	-	August 1972
Auburn University	-	November 1974

Current Annual Level: $49,500

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1975

Ecology Studies Section

CASE WESTERN RESERVE UNIVERSITY (NO1-CP-43201)

Title: Mortality experience of children who were inadvertently inoculated with SV-40 very early in life.

Contractor's Project Director: Eli Gold, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To determine cancer risk among children who received polio-vaccine containing SV-40 at birth.

Methods Employed: The mortality experience of 1,077 mainly Negro children, born in Cleveland, Ohio, between December 1959 and May 1962 and inoculated with SV-40 shortly after birth, will be followed through time. The location and name of each child are determined annually. Deaths are discovered through the use of a continuously up-dated census of Cleveland school children and from the vital records of the State of Ohio and other states to which the children have migrated. The names in the study group are compared against an alphabetic file of all childhood cancer deaths in the U.S. maintained in the Epidemiology Branch, NCI. The frequency of cancer deaths in the study group will be compared with the expected cancer mortality for children in the State of Ohio.

Major Findings: As yet there have been no cancers or other disorders in the study group attributed to SV-40. An interim report of the findings was published in Science 167: 59-60, 1970.

Significance to NCI Program and Bio-medical Research: Previous investigations by the Epidemiology Branch of school-age children who were inadvertently inoculated with SV-40 will be extended. The known importance of neonatal exposure in the causation of many viral neoplasms of animals suggests the possibility that the cancer mortality of children inoculated at birth might be different from that of children exposed at school age. The present study will extend our present knowledge in this area.

Proposed Course of Project: Since neoplasms with a latent period longer than 12 years could not be detected by the current follow-up, surveillance of the children will continue to be maintained.

Date Contract Initiated: March 9, 1964

Current Annual Level: $9,251

CONTRACT NARRATIVE
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1975

Ecology Studies Section

HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH (NO1-CP-33326)

Title: Epidemiology and Etiology of Breast Cancer

Contractor's Project Director: Philip Cole, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To evaluate the hypothesis that the estrogen profile in young women is a determinant of subsequent breast cancer risk.

Methods Employed: During the current contract year several evaluations of the estrogen fraction hypothesis were carried out: 1) a family study, involving analyses on daughters of breast-cancer patients; 2) a European study, involving three comparisons of high-risk and low-risk populations (Scandinavia vs. Central Europe, Finland vs. other Scandinavian countries, and the Netherlands vs. Yugoslavia and Greece); 3) a degradation study of stored frozen urine to determine the stability of estrogen fractions; 4) an analysis of etiocholanolone and pregnanediol excretion levels, and their correlations with breast-cancer risk and with patterns of estrogen excretion.

Major Findings: Preliminary analyses of data from the European study support the hypothesis that the estriol proportion is inversely related to breast-cancer risk. The results are in the same direction as earlier contrasts of women from North America vs. Asia, and Hawaiian women of Asian descent vs. Asian women in homelands and Hawaiian women of Caucasian descent. All studies to date have shown parallels between the population's breast-cancer risk and the urinary estrogen profile -- in the direction predicted by the hypothesis.

Preliminary results also suggest that, contrary to the findings of Bulbrook, etiocholanolone excretion is directly correlated with excretion of estrone and inversely with the estriol proportion. These results agree with the findings, as yet unpublished, that etiocholanolone levels are directly related to breast-cancer risk. In addition, a consistent and positive relationship has been found between breast-cancer risk and pregnanediol levels, although this measure is a crude indicator of progesterone activity.

Significance to NCI Program and Bio-medical Research: Epidemiologic characteristics of breast cancer are consistent with hormonal determinants, which can be best evaluated by joint epidemiologic-laboratory investigations of the type planned by this contract.

Proposed Course of Project: All work should be completed during the fifth and final contract year, 1975-76.

Date Contract Initiated: June 23, 1971

Current Annual Level: $78,382

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1975

Special Cancer Studies Section

ALBANY MEDICAL COLLEGE OF UNION UNIVERSITY (NO1-CP-12426)

Title: Study of Histopathologic Epidemiology of Childhood Cancer

Contractor's Project Director: Dr. J. N. P. Davies

Project Officer (NCI): Robert W. Miller, M.D.

Objectives: To carry out an international study of the incidence and frequencies of childhood cancer and to determine the causes of the striking variation in incidence from one country to another.

Methods Employed: Data on all childhood cancers have been collected from about 20 different countries. This statistical information is being analyzed by cell type and compared with data from other centers. A histologic review of the tumor types is being carried out when specific neoplasms may provide a basis for comparison, such as ovarian tumors and lymphomas which vary greatly from country to country. The principal investigator requests blocks or sections of certain tumors for this purpose.

Major Findings: The original hypothesis, that childhood cancers vary from one country to the next, as to cell type, has proven correct. A prime example of this variation is the very low risk of Ewing's tumor among Blacks in the United States and in Africa. The results have stimulated interest in childhood cancer among the contributing centers, some of which have in consequence published reports of their findings. Also, interest at WHO was stimulated, to the extent that an expert panel was convened on childhood cancer in Geneva in December 1972 to recommend a program to improve care and research concerning childhood cancer. Twenty-one recommendations were made, and efforts are now being made to implement them locally, nationally and internationally.

Significance to NCI Program and Bio-medical Research: Marked variations in the frequency of cancer according to race and geographic origin provide opportunities to identify genetic and environmental factors in etiology. The Epidemiology Branch has been involved in epidemiologic studies of childhood cancer in the U.S. The detection and clarification of international variation in childhood cancer should increase the opportunities for generating etiologic leads. The proposed histopathologic studies should play a very integral part in illuminating these leads.

Proposed Course of Project: The data collection has terminated and there is no further need for histological study. Support for the pathology study in Albany will be discontinued on June 23, 1975.

Date Contract Initiated: June 24, 1971

Current Annual Level: $22,250

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1975

Ecology Studies Section

NATIONAL ACADEMY OF SCIENCES (NO1-CP-43202)

Title: Epidemiologic Studies in Etiology of Cancer in Veterans

Contractor's Project Director: Gilbert W. Beebe, Ph.D. and Seymour Jablon, M.A.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D. and Robert W. Miller, M.D.

Objectives: To develop and conduct a broad program of epidemiologic studies in cancer among veterans.

Methods Employed: The Epidemiology Branch of the National Cancer Institute and the Follow-up Agency of the National Academy of Sciences will develop an epidemiology program designed to make efficient use of the military-veteran population, utilizing medical, demographic, and environmental observations of veterans ascertained through facilities of the Veterans Administration and supplemented by mortality data. The following activities were carried out during the past year: 1) A search for risk factors in Hodgkin's disease by case-control survey of 650 men under age 31 with at least 1 year of duty prior to diagnosis in a military hospital, 1950-68. This project is in the analysis phase, nearing completion after delays in data collection. 2) A case-control study of childhood cancer to evaluate prenatal radiation exposure, utilizing the childhood cancer mortality registry of the Epidemiology Branch, NCI, and pregnancy records available in military facilities. The record search for births in military hospitals has been completed in all but 4 states; approximately 1,300 births have been identified, and the clinical records of 550 cases have been abstracted so far. 3) A follow-up study of World War II veterans with certain conditions that may affect the risk of cancer, such as splenectomy, allergies, orchidopexy, nephrectomy, and alcoholism. Over 4,300 deaths have been reported in this group, and work is continuing to determine the specific causes of death. 4) An evaluation of familial factors in cancer by means of a Twin Registry of 16,000 pairs of veteran twins, with questionnaires recently sent to living co-twins of those with any form of cancer. Replies have been received from about 60% of the group and analysis will be made soon.

It is likely that proposals for additional studies will be submitted during the coming year after detailed protocols have been discussed.

Major Findings: Since this contract was initiated, reports have been published on the lack of a relationship between cancer and histories of infectious mononucleosis and exposure to yellow fever vaccine containing avian leukosis virus. In press is a report on the risk of respiratory cancer in World War I veterans exposed to mustard gas.

Significance to NCI Program and Bio-medical Research: The Epidemiology Branch, NCI, is concerned with studies to identify and clarify etiologic factors in cancer. These objectives can be more readily achieved by coordinating efforts with the exceptional resources and competent staff of the Follow-up Agency.

Proposed Course of Project: The various studies will continue into the next contract year, and new projects will be identified to clarify risk factors in cancer.

Date Contract Initiated: June 28, 1971

Current Annual Level: $102,000

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1975

Office of the Branch Chief

NATIONAL ACADEMY OF SCIENCES - NATIONAL RESEARCH COUNCIL (N01-CP-40044)

Title: Epidemiologic research on radiogenic cancer

Contractor's Project Director: LeRoy Allen, M.D.

Project Officer (NCI): Robert W. Miller, M.D.

Objectives: To determine the frequency and nature of cancer among atomic-bomb survivors in relation to radiation dose.

Methods Employed: The Atomic Bomb Casualty Commission has had clinics in Hiroshima and Nagasaki since 1948. Cancer research will continue through a) bi-annual clinical examinations of about 20,000 survivors with a wide range of radiation dosage, b) studies of death certificates for about 100,000 people who were in either of the cities at the time of the explosions, and c) a pathology program relating to both of these study samples. In addition, under the contract, citywide tissue registries are being established under the aegis of the Hiroshima Medical Association and Nagasaki University and one-year visiting scientist programs are offered to Japanese faculty members in Departments of Epidemiology, who consult the program for about 2 months in the United States before spending the balance of the year at the Hiroshima ABCC engaged in cancer epidemiology.

Major Findings: Under the contract, none as yet.

Significance to NCI Program and Bio-medical Research: The contract will permit continuation of cancer research which has revealed that radiation in sufficient dose can induce in man several forms of leukemia, thyroid cancer, lung cancer and breast cancer. An increase in stomach cancer is now suspected, but more cases must be accumulated before the evidence is conclusive. The ABCC findings do not support claims, based on studies of low-dose diagnostic irradiation, that all forms of childhood cancer are increased by intrauterine exposure. A recent finding at ABCC that persons exposed to the bomb before 10 years of age are showing a marked increase in various forms of neoplasia at 16-33 years of age, indicates that this cohort needs special attention to determine how long elevated cancer rates persist. In addition to the information derived about radiogenic cancer, this contract opens an opportunity for greater interaction between Japanese and American cancer epidemiologists at ABCC and in general.

Date Contract Initiated: February 29, 1972

Current Annual Level: $184,763

CONTRACT NARRATIVE
EPIDEMIOLOGY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1975

MELOY LABORATORIES, INCORPORATED (NO1-CP-43216)

Title: Viral Transformation of Cells from Persons at High Risk of Cancer

Contractor's Project Director: Mr. Michael M. Kaplan

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D. and William A. Blattner, M.D.

Objectives: To determine if sensitivity to viral transformation of fibroblasts identify persons at high risk of cancer.

Methods Employed: Studies by Todaro and his associates have revealed that skin fibroblasts from some individuals at high risk of cancer exhibit increased sensitivity to transformation by several oncogenic viruses, including SV-40. Sensitivity to transformation is evaluated by a fluorescent antibody technique which measures SV-40 induced T-antigen production in skin fibroblast culture.

Major Findings: A manuscript dealing with an improved method of cell fixation will be published in the Spring of 1975. Results of experiments performed to determine sources of variability in the assay are to be reported in the Spring meetings of the Federation American Society of Experimental Biology. The results of these experiments have led to development of a more reproducible and reliable assay system. Over the last year a complete inventory of cell lines has been stored on computer tape so that information about these cell lines can be easily accessed. The total number of cell lines now in storage is over 2,000.

Significance to NCI Program and Bio-Medical Research: The Epidemiology Branch of the National Cancer Institute is concerned with studies to identify groups at high risk of cancer. This objective will be fostered by joint epidemiologic-laboratory investigations of the type planned by this contract, in collaboration with other investigators in the Viral Oncology Area of NCI. The Epidemiology Branch will continue to make this laboratory resource available to other members of the research community who have patients appropriate for study utilizing this technique. The findings should be of value not only in etiologic research, but also clinically in counseling and prevention.

Proposed Course of Project: Experiments are underway to evaluate individuals known to be at high risk for malignancy to confirm the previously described correlation with transformation susceptibility. On completion of these studies full scale testing of other high risk groups compared to normal controls will be undertaken. Observations in this laboratory as well as others have indicated that there may be transformation "enhancing factors" present in the cells which are used to manufacture virus for testing. Experiments are underway

to evaluate this observation as a potential source of variability in the assay and the possibility that such "enhancing factors" might prove useful in the development of a more refined assay procedure.

Date Current Contract Initiated: October 1, 1974.

Current Annual Level: $213,144.

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1975

UNIVERSITY OF MINNESOTA, MINNEAPOLIS, MINNESOTA (NO1-CP-33357)

Title: Immunodeficiency -- Cancer Registry

Contractor's Project Director: John H. Kersey, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: Study the frequency and possible determinants of malignancy in immunodeficient patients.

Methods Employed: Through the cooperation and goodwill of immunologists in many of the specialized immunology centers throughout the world, clinical information and occasionally biologic specimens from immunodeficient patients who have developed cancer are sent to the Registry for tabulation and analysis. In addition, in this past year the data collection phase of a case-control study of cancer in immunodeficient children has been initiated. Epidemiologic and laboratory information are being abstracted from the hospital charts of immunodeficient children who developed a malignancy and matched control immunodeficient children who did not.

Major Findings: New findings in the past year concern special analyses of the experience in adults. There is an excess of lymphoreticular malignancies similar to that previously noted for children. There also appears to be an excess of stomach cancer in adults. No other tumors were notably excessive in this group.

Significance to NCI Program and Bio-medical Research: Patients with altered immunologic state have demonstrated excess risks of malignancy several hundred times that of the general population. Study of the determinants of these excesses have a high probability of shedding light on immunologic aspects of cancer etiology.

Date Contract Initiated: July 1, 1974

Current Annual Level: $23,133

UNIVERSITY OF PADUA (NO1-CP-3-3359)

Title: Collection of Human Tissue Specimens

Contractor's Project Director: Professor Giovanni Dogo

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To determine the role of genetics versus the role of environmental factors in the high incidence of gastric cancer among several groups resident in the several towns in the Dolomite region of Italy. To examine and take medical histories of individuals and families in this group. To collect sera and biopsies for further testing from this high risk group as warranted by risk factors. To document the incidence of cancer in the group.

Major Findings: Skin biopsies from persons in three towns have been cultured to produce fibroblastic cells. Geneologies on each subject have been compiled going back 4-5 generations. These have been analyzed by computer to calculate coefficients of relationship and inbreeding. The cell cultures were highly susceptible to transformation by SV 40 virus; but the transformation rates did not correlate with coefficients of inbreeding, nor were high transformers more closely related to each other than to low transformers.

Cancer deaths were abstracted from town records from 1935 to 1969. There were 637 deaths, of which 97 were from cancer. There was no apparent temporal trend in the distribution of cases. Seventy percent of cancer deaths were listed as stomach cancer. Stomach cancer clustered strongly within families, while other types did not. Biopsy subjects from high stomach cancer families did not have high SV 40 transformation rates.

Significance to Biomedical Research and the Program of the Institute: This research may help determine the relative contributions of environment and genetics to the etiology of stomach cancer.

Proposed Course: To determine what proportion of gastric cancer cases were diagnosed histologically. To search the records of other towns to determine if there was a similar clustering of gastric cancer. To take and culture skin biopsies as warranted by developments with SV 40 testing.

Date Contract Initiated: October 27, 1964

Current Annual Level: $13,166

ANNUAL REPORT

CARCINOGENESIS PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE

July 1, 1974 through June 30, 1975

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1. OVERVIEW

A. INTRODUCTION

A vast majority of cancers, affecting people throughout the world, appears to be caused by chemical and physical agents. Rapidly growing awareness of this evidence has placed strong emphasis on the need to define carcinogenic hazards and on the opportunities for prevention. Good evidence has been obtained on the following roles of chemical and physical agents: (1) tobacco smoking in the causation of cancers of the lung, larynx, oral cavity, esophagus, bladder, and possibly other sites; (2) Ultraviolet light and certain chemical exposures in the causation of cancers of the skin; (3) ionizing radiation in the causation of leukemias, cancers of the lung, thyroid, and several other neoplasms; (4) alcoholic beverages in the causation of cancers of the larynx, esophagus, and other neoplasms; (5) environmental changes, as revealed by the study of migrant populations, in the causation of cancers of the large bowel, stomach, and other sites; (6) occupational exposures on the causation of cancers at many sites (lung, bladder, skin, liver, etc.); (7) nutritional factors in the development of cancer at various sites; and (9) hormonal preparations in the development of cancer at several sites. In addition, high susceptibility host factors have been defined for several population groups.

The knowledge thus far acquired on cancer pathogenesis can already be effectively used to prevent a large proportion of cancers in our population, and action toward this goal can be taken by each individual by adopting certain habits which are clearly associated with a lower cancer risk--no smoking, no drinking of hard liquors, adoption of a "prudent" diet, protection from ultraviolet light for individuals of fair skin complexion, protection from occupational exposure to carcinogens by seeking adequate information on risks and adoption of protective measures.

Societal decisions, beyond individual control, are involved in the development of additional levels of prevention requiring changes in technology, development and adoption of less hazardous products, and regulatory control of exposures. An objective, well-documented scientific basis is needed for such decisions by various segments of society--government, industries, labor, and consumer and environmental protection groups. It is one of the responsibilities of a carcinogenesis program within the Federal health research agency, NIH, to provide such a basis of scientific knowledge and documentation.

A major societal decision is also involved in establishing priorities for developing and supporting the basic and applied research needed to provide a more extensive knowledge and documentation on the substances and exposures that cause cancers, on the factors which determine our susceptibility to them, and finally, on the biologic and chemical mechanisms that can be "exploited" to prevent, inhibit, or delay the onset of cancers in the population.

Protecting the population from chemical and physical carcinogenic hazards and their effects has been clearly recognized as an urgent and essential task in our society, and a high national priority for this mission has been forcefully indicated by the Cancer Act, by Congress, and by the Administration. The Carcinogenesis Program continues to be developed to serve towards the accomplishment of these national goals.

This NCI Program originated in the Carcinogenesis Studies Branch, established in 1961 as part of the Field Studies Area. In 1966 the Carcinogenesis Program was organized under the Office of the Associate Scientific Director for Carcinogenesis within the newly established Etiology Area. In 1968 a Program plan was adopted after extensive consultation with leading scientists in the field. New intramural research branches were established and a major contract-supported collaborative program was developed through several segments. In 1972 the Etiology Area became the present Division of Cancer Cause and Prevention. The Tobacco Research Segment of the Carcinogenesis Program was transferred to the Division in 1973 and expanded as a separate Smoking and Health Program.

The Carcinogenesis Program has developed into a complex effort based on the collaboration of some 200 laboratories in different institutions including our intramural research laboratories, all devoted to the study of carcinogenesis and cancer prevention, all committed to a teamwork approach to our field. Carcinogenesis research has developed into a complex multidisciplinary approach where investigators of many different disciplines work closely together--human and experimental pathologists, chemists, toxicologists, epidemiologists, occupational health specialists, biochemists, enzymologists, immunologists, cell biologists, molecular biologists, geneticists, etc. The Program is characterized by the integration of basic research of high scientific quality and applied targeted research oriented toward specific disease problems, supported by appropriate resources.

The effectiveness of the Program is determined not only by the level of financial support provided to it, but above all by the availability of a staff of high scientific competence, totally dedicated to its imposing task, and by the collaboration of a large, outstanding scientific community in universities and research institutes as well as in industrial and commercial laboratories.

The Program has developed a planning and review structure for collaborative research, which provides the invaluable help of a highly qualified group of scientific leaders and advisors from outside institutions for the critical development of our activities, to whom we are deeply grateful. Much of the success of the Program is due to the collaborative constructive spirit with which scientists in over 150 institutions across the country and abroad have joined forces in this exciting fight against the induction of cancer.

Recognition is due to the Carcinogenesis Program staff to whose extra-ordinary, invaluable, and untiring efforts this Program owes its development. In spite of strict limitations in personnel positions, an excellent, highly

productive intramural research program has been maintained together with a rapidly expanding collaborative program; both were made possible by the highly dedicated, often strenous commitment of the members of the staff.

Major national policy decisions, concerning the prevention of exposure of the population to carcinogenic agents, have been made by agencies of the Federal Government in this fiscal year, on the basis of scientific documentation and evaluation largely provided by the NCI's Carcinogenesis Program: notable among these are the decision of the Administrator of the Environmental Protection Agency to suspend the use of the pesticides Aldrin/Dieldrin on the basis of conclusive experimental evidence of carcinogenicity and lack of demonstrated overriding societal needs (a decision upheld by the U.S. Court of Appeals); the decision of the Occupational Safety and Health Administration (Department of Labor) to require a standard of exposure to vinyl chloride at the lowest level that technology can currently achieve; the position of the National Institute of Occupational Safety and Health that human hazards ought to be investigated and exposure minimized for chemicals shown conclusively to be carcinogenic by adequate animal bioassays.

The continuous data collection, review, and study of the current state of knowledge in carcinogenesis by Program staff and highly qualified outside consultants, has so far not altered the conclusion that no level of exposure to a carcinogen should be considered safe for man. This position was again recently confirmed by the Secretary of Health, Education and Welfare, with the following statement: "At present, the Department of Health, Education, and Welfare lacks the scientific information necessary to establish no-effect levels for carcinogenic substances in animals in general and in man in particular. In the absence of such information, we do not believe that detectable residues of carcinogenic animal drugs should be allowed in the food supply."

The Conference on Occupational Carcinogenesis, held at the New York Academy of Sciences on March 24-27, 1975, and co-sponsored both by the NCI and by the National Institute for Occupational Safety and Health, proved to be a milestone in this field, since general consensus was expressed on the need for maximal prevention from exposure to carcinogens--including those identified through animal tests and before human cancers already occur--and this view was expressed by speakers from various Federal agencies, from industry, from labor, from public interest groups, and from the scientific community.

At this Conference, the Associate Director for Carcinogenesis presented a paper on the role of the NCI in the Panel entitled "Prevention of Occupational Cancer--Toward an Integrated Program of Governmental Action", which concluded with the following statement:

> "The current state of our knowledge of chemical carcinogenesis is still such that we are unable to determine a safe level of exposure to a carcinogen for man. Preventive approaches are, therefore, largely dependent on safer technological alternatives

and on the best available technologies to minimize or, if possible, eliminate exposure.

"The societal implications of such technological alternatives may be of major importance, as for example in the present concern on the choice of alternate energy sources.

"Society has to arrive at these difficult choices with full cognizance of their health implications.

"Cancer--in the last quarter of the twentieth century--can be considered as a 'social disease', a disease whose causation and control are rooted in the technology and economy of our society. The prevention of cancer is largely an attainable goal, but it requires the coordinated effort of our society in its many components: government, the scientific community, industry, labor, and qualified public opinion.

"The NCI is committed to continue and expand its efforts in this vital part of the National Cancer Program."

B. PROGRAM PLANS

Program Plans on Chemical Carcinogenesis and Prevention of Cancers - The plan was developed in 1968 through an extensive series of planning sessions conducted by Dr. Carl G. Baker, then Scientific Director for Etiology, Dr. Umberto Saffiotti, Associate Scientific Director for Carcinogenesis, and Mr. Louis M. Carrese, with the participation of Dr. Abraham Cantarow, and Mr. Richard A. Terselic. The Report of the Discussion Groups on Chemical Carcinogenesis, coordinated by Dr. Cantarow for the National Advisory Cancer Council, served as an excellent basis for outlining the research needs in the field of carcinogenesis. The convergence technique was used to identify the major objectives and the linear array of subsequent phases leading to their implementation. An outline of the program plan is attached (see Chart) and its main phases are discussed below. The program is articulated in a number of segments. The first segment deals with (a) the epidemiologic identification of population groups showing different risks to different types of cancer and (b) to the characterization of such individuals as to their environmental factors and their biological and functional parameters. The program then develops the following distinct approaches:

(1) identification of carcinogenic activity of selected chemicals by bioassay,

(2) identification, development, and selection of biological models for carcinogenesis bioassays as well as for the characterization of carcinogenesis processes, and

(3) identification of processes required for the carcinogenic action of selected agents as target points for corrective measures.

The first approach requires an initial phase devoted to the identification and selection of chemical agents to be entered in the bioassay systems. The development and implementation of bioassay procedures requires a great deal of accurate standardization so that selected chemical agents will be tested through a battery of precisely defined and reproducible bioassay systems, each well characterized for its sensitivity to specific carcinogenic effects.

After appropriate monitoring for relevance and for program needs, the results of the first phases of the program lead to the identification and possibly to the development of corrective measures based, on one hand, on modifications of the environment and, on the other hand, based on modifications of individual target points in the process of carcinogenesis, identified by previous studies. These target points can be visualized as taking place at any one of the several steps required for the process of carcinogenesis to be completed. They include the following: (1) penetration of the chemical agent into the whole organism; (2) transport, retention and elimination of the chemicals to tissues (molecular logistics); (3) metabolic pathways to proximate carcinogen; (4) cell and tissue factors required for the previous steps; (5) penetration into target cells and interaction with cell constituents; (6) neoplastic transformation and conditioning factors; and (7) growth regulation of transformed cells.

The results thus obtained are in turn appropriately monitored for relevance; the program then considers the development of corrective measures and ultimately suggests application of such corrective measures through the human environment or through the human subject. The last phase of the program deals with the evaluation and validation of the results of such corrective measures in the human situation and is expected to lead to the recognition of decreased cancer risks and in the populations originally studied in Phase I. Each of the major phases of the program requires organization of data banks, as a necessary source of information to be used for the monitoring of the whole program.

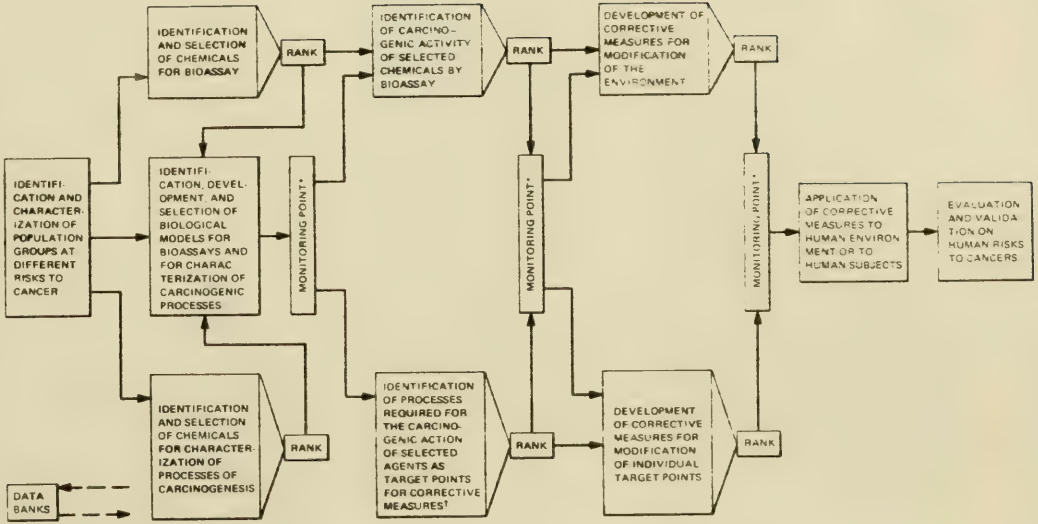
The program is aimed at a comprehensive attack to the problem of chemical carcinogenesis. It recognizes the need for very extensive bioassay efforts but also recognizes that the accumulation of information on the carcinogenicity of chemicals in animal systems will not provide a direct extrapolation to the hazards in man. Moreover, it is predictable that a large number of chemical carcinogens will be difficult to remove and, therefore, will remain in the environment of man for a long time to come even after being identified (e.g. combustion products, cigarette smoke, natural food contaminants such as mycotoxins, metals and other air pollutants). It is, therefore, imperative that an effort be made not only to identify and possibly remove an increasing number of carcinogens from the environment, but also, and concurrently, to identify steps in their mode of penetration into the organisms and the target tissues and in their critical interaction with cell constituents so that these steps could be exploited as target points for inhibitory or protective measures. The considerable development of refined technical methods presently available for the identification of these critical steps in carcinogenesis by studies in analytical chemistry, biochemistry, cell biology,

molecular biology, and immunology can now be brought to bear on our ability to develop useful, protective measures against the ultimate effects of the carcinogenic process. The identification of critical parameters required for the carcinogenic action of certain groups of chemicals, obtained in animal studies, also represents the only direct link that can be applied to the study of the susceptibility of man to comparable exposure conditions.

For example, the establishment of a strong correlation between the enzymatic activation of certain chemicals to the proximate carcinogen by means of given groups of enzymes, such as the microsomal aryl hydroxylase for polynuclear hydrocarbons may lead to the identification of groups of individuals as being highly susceptible to certain specific carcinogenic exposures, pharmacologic inhibition of some of these enzyme systems may lead to a marked decrease of the cancer risk for such individuals. As other examples, analogous approaches can be made at the level of the control of protein synthesis in differentiation-dependent carcinogenic mechanisms (e.g. hormone-dependent tumors and vitamin A-dependent tumors) and at the level of the immunological control of the growth of transformed cells. A broad front of attack can thus be developed against the sequence of events required for the carcinogenic process to lead to the establishment of progressively growing invasive tumors.

The effective development of such studies depends in part on the availability of appropriate animal models, capable of reproducing tumor types analogous to those observed in man. The development of appropriate biological models is particularly important for the establishment of selectively sensitive bioassay systems. An important phase of the program consists in the selection and standardization of bioassay systems that could be reproduced in several laboratories under strictly controlled standardized conditions. This is an essential requirement if we want to collect and analyze large series of data on carcinogenesis tests. For this purpose, a detailed data retrieval and analysis system for carcinogenesis studies is required.

The present overall Program Plan in Chemical Carcinogenesis and Prevention of Cancers constitutes the basis for the development of detailed operational plans aimed at the implementation of each of its phases. Special emphasis has been given to special plans for carcinogenesis in the lung, colon, pancreas, and other sites, and to the development of short-term bioassay methods for carcinogens using methods of mutagenesis and transformation of cells in culture.


IDENTIFICATION AND SELECTION OF CHEMICALS FOR BIOASSAY
RANK
IDENTIFICATION OF CARCINOGENIC ACTIVITY OF SELECTED CHEMICALS BY BIOASSAY
RANK
DEVELOPMENT OF CORRECTIVE MEASURES FOR MODIFICATION OF THE ENVIRONMENT
RANK
IDENTIFICATION AND CHARACTERIZATION OF POPULATION GROUPS AT DIFFERENT RISKS TO CANCER
IDENTIFICATION, DEVELOPMENT, AND SELECTION OF BIOLOGICAL MODELS FOR BIOASSAYS AND FOR CHARACTERIZATION OF CARCINOGENIC PROCESSES
MONITORING POINT*
MONITORING POINT*
MONITORING POINT*
APPLICATION OF CORRECTIVE MEASURES TO HUMAN ENVIRONMENT OR TO HUMAN SUBJECTS
EVALUATION AND VALIDATION ON HUMAN RISKS TO CANCERS
IDENTIFICATION AND SELECTION OF CHEMICALS FOR CHARACTERIZATION OF PROCESSES OF CARCINOGENESIS
RANK
IDENTIFICATION OF PROCESSES REQUIRED FOR THE CARCINOGENIC ACTION OF SELECTED AGENTS AS TARGET POINTS FOR CORRECTIVE MEASURES†
RANK
DEVELOPMENT OF CORRECTIVE MEASURES FOR MODIFICATION OF INDIVIDUAL TARGET POINTS
RANK
DATA BANKS

*Monitoring points. According to program relevance and needs, determine further action and channel through next phases

†Through studies on the penetration of the agents into the organism, their transport, retention, and elimination, their metabolic conversion to the proximate carcinogen, the cellular and tissue factors required for such processes, the penetration into target cells and interaction with cell constituents, the neoplastic transformation, and the factors required for the growth of transformed cells into tumors

CHEMICAL CARCINOGENESIS AND PREVENTION OF CANCER (SUMMARY OF PROGRAM PLANS).

2. ORGANIZATIONAL STRUCTURE AND MANAGEMENT

The organization of the Carcinogenesis Program is presently divided into two major components: intramural program and collaborative program. The former is articulated in the Office of the Associate Director for Carcinogenesis and in six branches, each with several sections and units. The latter is articulated into eight segments. Each segment is directed by a senior staff member, assisted by a manager; other members of the staff serve as project officers on individual contracts. Each segment is assisted by individual ad hoc advisors from outside NCI. Three technical peer review groups have been established.

A. INTRAMURAL PROGRAM

Office of the Associate Director for Carcinogenesis (U. Saffiotti, M.D., Associate Director; J. A. Cooper II, Ph.D., Deputy Associate Director; C. R. Warner, Ph.D.):

Office of the Scientific Coordinator for Carcinogenesis at the NCI's Frederick Cancer Research Center (R. R. Bates, M.D., Scientific Coordinator; B. Chen, Ph.D., J. Idoine)

Biology Branch (H. J. Rapp, Sc.D., Chief; T. Borsos, Sc.D., Associate Chief):

Cellular Immunity Section (B. Zbar, M.D., Head; R. Bast, M.D., R. Harmel, M.D., T. Ogura, M.D.[1], T. Okuda, M.D.[1])
Cytogenetics and Cytology Section (J. A. DiPaolo, Ph.D., Head; P. Donovan, C. Evans, M.D., B. Myhr, Ph.D., R. Nelson, N. Popescu, Ph.D.)
Immunochemistry Section (T. Borsos, Sc.D., Head; A. Bernard, M.D.[1], M. Boyle, Ph.D.[1], B. Hooks, Ph.D., S. Ohanian, Ph.D., M. Segerling[1], M. Xuma, M.D.[1])
Tumor Antigen Section (E. Leonard M.D., Head; L. Boumsell, M.D.[1], M. Lett-Brown, Ph.D.[1], M. Meltzer, M.D.)

Carcinogen Bioassay and Program Resources Branch (N. P. Page, D.V.M., Chief; T. Cameron, D.V.M., C. Cueto, Jr., Ph.D., M. Linhart, S. Siegel, Ph.D.)

Carcinogen Metabolism and Toxicology Branch (E. K. Weisburger, Ph.D., Chief; S. Gothoskar, Ph.D.[1])

Metabolic Studies Section (E. K. Weisburger, Ph.D., Acting Head; R. Evarts, D.V.M., Ph.D., P. Grantham, L. Poirier, Ph.D.)
Analytical Chemistry Unit (L. K. Keefer, Ph.D., Acting Head; W. Gaffield, Ph.D.[1], P. Roller, Ph.D.)
Bacterial Metabolism Unit (S. Silverman, Ph.D., Acting Head)

[1]Guest Worker

Chemistry Branch (H. V. Gelboin, Ph.D., Chief):

Cell Growth Regulation Section (J. P. Bader, Ph.D., Head; R. Harris, Ph.D., D. Hatfield, Ph.D., T. Okazaki, M.D., Ph.D.[1])
Molecular Carcinogenesis Section (H. V. Gelboin, Ph.D., Acting Head; N. Nemoto, Ph.D.[1], J. Selkirk, Ph.D., F. Wiebel, M.D.)
Nucleic Acids Section (C. W. Dingman, M.D., Head; R. Day, M.D., T. Kakefuda, M.D., T. Kakunaga, Ph.D.)
Protein Section (A. C. Peacock, Ph.D., Head; S. Bunting, M. Green, Ph.D., K. Nishinaga, Ph.D.[1], J. V. Pastewka)

Experimental Pathology Branch (U. Saffiotti, M.D., Chief; R. A. Squire, D.V.M., Ph.D., Associate Chief; R. Yamamoto, Sc.D.):

In Vitro Pathogenesis Section (S. Yuspa, M.D., Head; W. D. Edwards, Ph.D.[1], K. Elgjo, M.D.[1], H. Hennings, Ph.D., D. Morgan, U. Lichti, Ph.D.)
Perinatal Carcinogenesis Section (J. M. Rice, Ph.D., Head; S. Joshi, Ph.D.)
Tumor Pathology Section (R. A. Squire, D.V.M., Ph.D., Head; M. Barrett-Deringer, Ph.D., D. Goodman, V.M.D., C. Lingeman, M.D., M. Levitt, M.D. (T. Dunn, M.D. and H. L. Stewart, M.D., Consultants)
Ultrastructure Unit (C. C. Harris, M.D., Acting Head)

Lung Cancer Branch (M. B. Sporn, M.D., Chief; J. Smith):

Differentiation Control Section (L. De Luca, Ph.D., Head; J. Frot-Coutaz, Ph.D.)
Lung Cancer Pathogenesis Section (A. Frank, M.D., C. C. Harris, M.D., D. Kaufman, M.D., Ph.D., D. Reese, Ph.D., C. Van Haaften[1])

B. COLLABORATIVE PROGRAM

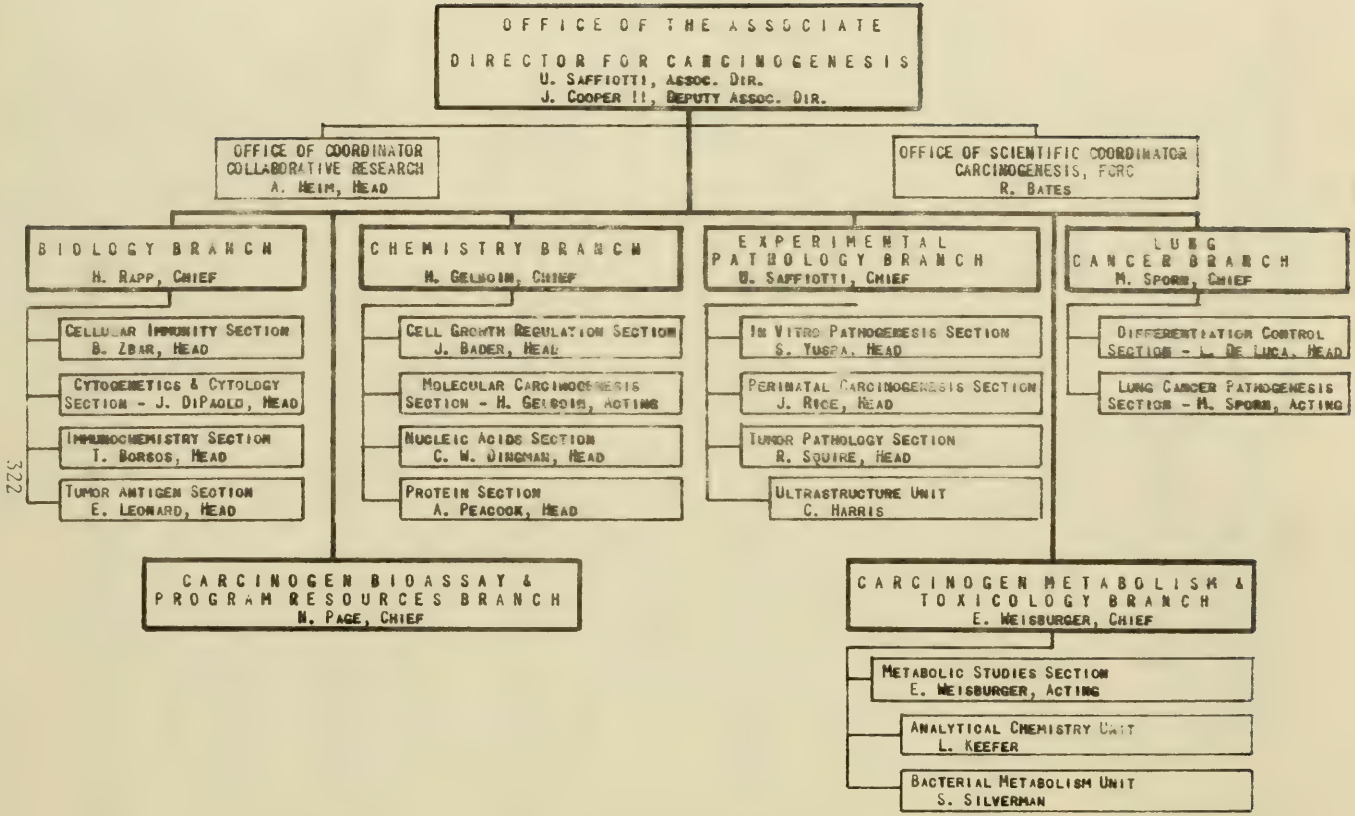
Office of the Associate Director for Carcinogenesis (U. Saffiotti, M.D., Associate Director; J. A. Cooper II, Ph.D., Deputy Associate Director; A. L. Heim, Ph.D., Coordinator for Collaborative Research)

Bioassay Operations Segment (N. P. Page, D.V.M., Director; J. Sontag, Ph.D., Manager)
Biological Models Segment (R. Pledger, Ph.D., Acting Director; R. Pledger, Ph.D., Manager)
Biology and Immunology Segment (H. J. Rapp, Sc.D., Director; V. Dunkel, Ph.D., Manager)
Carcinogen Metabolism and Toxicology Segment (E. K. Weisburger, Ph.D., Director; J. Sontag, Ph.D., Manager)

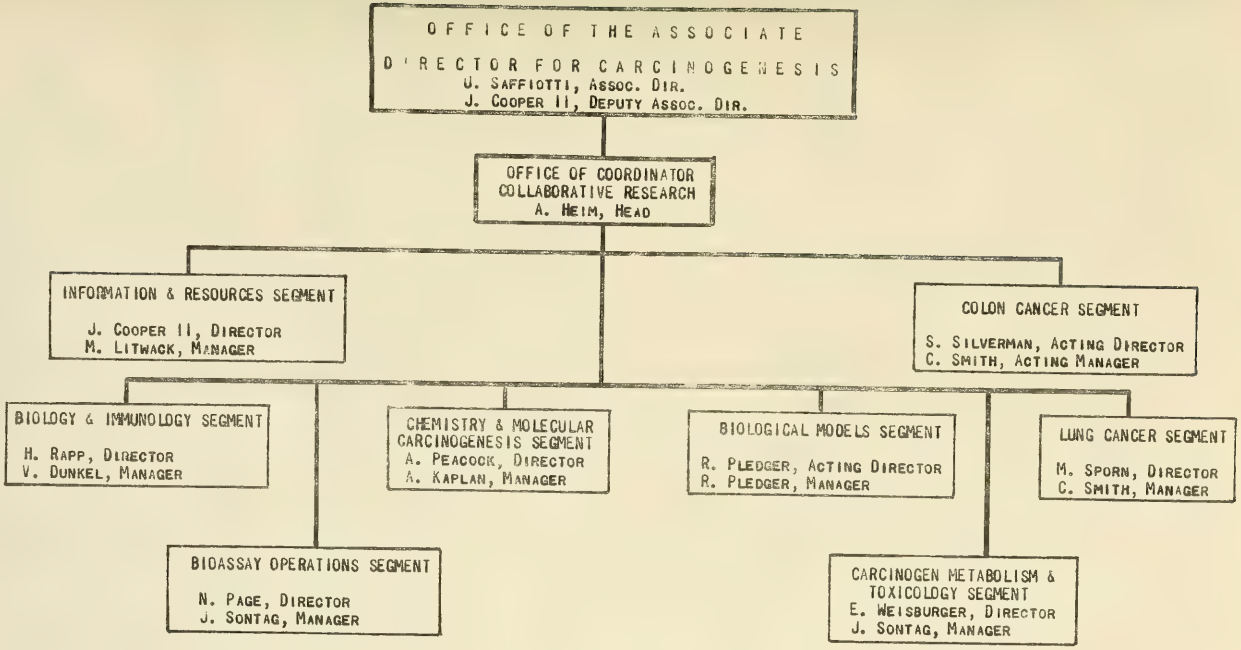
[1]Guest Worker

Chemistry and Molecular Carcinogenesis Segment (A. C. Peacock, Ph.D., Director; A. E. Kaplan, Ph.D., Manager)
Colon Cancer Segment (S. Silverman, Ph.D., Acting Director; C. Smith, Ph.D., Acting Manager[1])
Information and Resources Segment (J. A. Cooper II, Ph.D., Director; M. D. Litwack, Ph.D., Manager)
Lung Cancer Segment (M. B. Sporn, M.D., Director, C. Smith, Ph.D., Manager)

[1]A. E. Kaplan, Ph.D., served as Acting Manager from December 1972 to March 1975.



INTRAMURAL ORGANIZATION



COLLABORATIVE RESEARCH ORGANIZATION

C. MANAGEMENT AND REVIEW SYSTEM

Management of the Carcinogenesis collaborative Program has continued under the same basic philosophy as in previous years. The actual procedures for review and committee management, however, have been under constant change because of changing guidelines and regulations.

The philosophy that a strong science base is essential for effective management of a complex contract-supported collaborative program remains fundamental to the Program management activities. This was reflected in the use in previous years of segment advisory groups staffed by (1) segment directors drawn from the intramural staff who provide scientific leadership, (2) segment managers, who as scientist administrators provide management expertise and support, (3) senior intramural staff, actively engaged in intramural research, who also serve as project officers and who provide technical expertise and scientific guidance, and (4) outside consultants drawn from the scientific community who provide technical expertise, advice on Program direction, and review as a peer group. Departmental requirements for the reduction in numbers of formal committees made it necessary to abolish the eight segment advisory groups and to establish three new Carcinogenesis Program Scientific Review Committees A, B, and C. In order to insure the necessary extent and quality of outside scientific advice available at the segment level, review and advice has been obtained from outside consultants on an individual basis as required.

Segments continue to have responsibility for developing specific programs based on their fundamental objectives within the framework of the overall Carcinogenesis Program. As a basis for the implementation of its operational plans, each segment receives a preliminary budget apportionment from the Carcinogenesis budget. This assists with planning and program management and also forces priority choices to be made between continuing or expanding existing programs and initiating new activities. When new programs are initiated, requests for proposals (RFPs) are formulated at the segment level, discussed with outside advisors, reviewed by the Carcinogenesis Contract Program Management Group (CCPMG), and subsequently advertised to the academic and commercial communities. Proposals are reviewed by the segment with the assistance of scientific experts from outside the Program for technical merit and relevance to the segment program. In addition, extensive use has been made of the participation of ad hoc individual reviewers, and of mail reviews.

After adequate review, recommendations for initiation and support of a particular research program are made by each segment to the CCPMG, an advisory group of staff members which reviews proposed activities for overall priority and relevance within the total Carcinogenesis Program. Membership of this group consists of representatives of all the major elements in the Carcinogenesis Program: The Office of the Associate Director for Carcinogenesis, the branch chiefs from the intramural scientific staff, segment directors from collaborative program scientific staff, as well as a representative from the Division of Cancer Research Resources and Center who provides liaison with the grants program. Segment managers, contract specialists, the administrative officer, and appropriate project officers attend the meetings as observers and resource staff.

Over the past year a number of significant developments have taken place which have affected program management. One of these was the establishment and initiation of an NCI program on in vitro carcinogenesis. While it encompasses activities across the entire Institute, the coordination and management of the program are the responsibility of the Carcinogenesis Program. Because of the major impact of methods for carcinogenesis in cell cultures on the development of prescreening approaches to the identification of environmental cancer hazards, as well as on the elucidation of mechanisms of cancer induction and prevention, there was a need to start the program on a sound scientific basis, but also as rapidly as possible. The program needs to utilize presently known in vitro systems for neoplastic transformation by chemical and physical agents and also to develop and explore new approaches in an area of delicate, advanced research methodology. The coordination of the program is a formidable management challenge which had to be met without allocation of new scientific and management staff positions. The program was established directly in the Office of the Associate Director, under the management responsibility of Dr. V. C. Dunkel, who accepted this task in addition to her other management activities. Collaboration and scientific advice was obtained from members of the staff in the intramural program and from highly qualified outside consultants.

Another major development requiring additional management has been the introduction of Cancer Research Emphasis Grants (CREG) into the collaborative program as a funding mechanism to be used in addition to contracts. Details of implementation are still being developed but planning and background work have been required to date. Use of both grants and contracts in a planned effort has a great deal of constructive potential and presents the manager with more flexibility than before for encouraging good scientific participation in the program.

Workshops and conferences have played an important role in providing segments and the program with a mechanism for bringing the outside expertise of investigators and consultants into the planning process. Approximately 15 of these will have taken place by the end of the fiscal year. Prominent among these was the Third Annual Carcinogenesis Collaborative Conference held on February 2-5, 1975, in Orlando, Florida, with over 400 participants, the largest Annual Conference held so far. Much time and effort is required for the planning and conduct of a conference of this kind, and this was made possible only through the cooperation of the Carcinogenesis staff. The value of these workshops and conferences is evident from the interest and comment of participants who are anxious to learn about new work as it is in progress and are able to discuss methodology and data directly with others working on related projects in a concerted effort to solve complex problems. These group meetings represent an effective interaction of those investigators who participate in team work efforts.

The major challenge of managing a collaborative program of the present size and complexity lies in the fact that staff resources are not increasing while staff responsibilities are increasing very rapidly and regulations for committee management, requirements for more and more contract documentation, complexity of review procedures, involvement of the outside scientific community in planning and participating in all aspects

of the research are also increasing at an extremely rapid rate. This is coupled with the perennial problem of not knowing what the actual program budget is until late in the fiscal year.

The major management problem of the Carcinogenesis collaborative Program is the serious overextension of the staff. Despite ever increasing demands on time and documentation necessary to comply with an ever increasing and changing number of regulations and requirements, there is not sufficient staff to provide a manager for each segment or a secretary for each manager. This jeopardizes both the quantity and quality of the work which the staff can reasonably or even unreasonably do and results in a situation which needs immediate attention. The overextension of the staff applies also to the scientific planning and monitoring functions, which are severely impaired by the lack of available staff expertise in many critical program areas for project officers, reviewers, and program leader functions.

The abbreviated recounting of management activities intentionally omits any discussion of the efforts required for planning and obtaining approval of new programs, development of project plans, issuance of RFPs, establishing review committees, conducting site visits, assisting with negotiations, and providing guidance and assistance with daily operational problems. These management tasks are assumed to occur routinely but it should be stressed that they constitute major requirements of time and effort.

D. ADVISORY GROUPS AND CONSULTANTS

(1) The Carcinogenesis Contract Program Management Group (CCPMG) is a committee of staff scientists who provide review of proposed activities for priority and relevance within the Carcinogenesis Program. Membership of this group consists of representatives of all the major elements in the Program: The Office of the Associate Director for Carcinogenesis, the branch chiefs from the intramural scientific staff, segment directors from collaborative program scientific staff, as well as a representative from the Division of Cancer Research Resources and Centers.

Members: Dr. Umberto Saffiotti, Associate Director for Carcinogenesis, Chairman
Dr. Virginia Dunkel, Executive Secretary
Dr. Richard R. Bates, Scientific Coordinator for Carcinogenesis NCI's Frederick Cancer Research Center, OADC
Dr. John A. Cooper II, Director, Information and Resources Segment
Dr. Thaddeus Domanski, Program Director for Carcinogenesis, Division of Cancer Research Resources and Centers, NCI
Dr. Harry V. Gelboin, Chief, Chemistry Branch
Dr. Allen Heim, Coordinator for Collaborative Research, OADC
Dr. Herman Kraybill, Scientific Coordinator for Environmental Cancer, Office of the Director, DCCP
Dr. Norbert Page, Director, Bioassay Operations Segment
Dr. Andrew C. Peacock, Director, Chemistry and Molecular Carcinogenesis Segment
Dr. Richard Pledger, Acting Director, Biological Models Segment
Dr. Herbert J. Rapp, Director, Biology and Immunology Segment
Dr. Michael B. Sporn, Director, Lung Cancer Segment
Dr. Elizabeth K. Weisburger, Director, Carcinogen Metabolism and Toxicology Segment

(2) The Carcinogenesis Intramural Program Group (CIPG) provides a coordinating and planning function to the intramural program at the senior staff level. Since the vast majority of the intramural professional staff is now active in the planning and monitoring of the overall program, this group provides a vital interface between intramural and collaborative research programs. Membership of this group is limited to the staff of the Office of the Associate Director for Carcinogenesis, the Administrative Officers, and the six branch chiefs.

Members: Dr. Umberto Saffiotti, Associate Director for Carcinogenesis, Chairman
Dr. Richard Bates, Scientific Coordinator for Carcinogenesis NCI's Frederick Cancer Research Center, OADC
Dr. John Cooper II, Deputy Associate Director for Carcinogenesis
Mr. Damian Crane, Administrative Officer
Dr. Harry Gelboin, Chief, Chemistry Branch
Dr. Allen Heim, Coordinator for Collaborative Research
Dr. Norbert Page, Chief, Carcinogen Bioassay and Program Resources Branch

Dr. Herbert Rapp, Chief, Biology Branch
Dr. Michael Sporn, Chief, Lung Cancer Branch
Dr. Robert Squire, Associate Chief, Experimental Pathology Branch
Dr. Elizabeth Weisburger, Chief, Carcinogen Metabolism and Toxicology Branch

(3) The Carcinogenesis Program Scientific Review Committees A, B, and C provide expert peer review of proposals submitted to the Program. Based on an evaluation of the scientific merit of the proposed work and on an evaluation of the technical competence of the proposed staff, the Committees provide recommendations concerning the award of contracts or other appropriate support instruments.

Carcinogenesis Program Scientific Review Committee A

Members: Dr. Allen Heim, Executive Secretary
Dr. Howard A. Bern, Professor of Zoology and Research Endocrinology, University of California at Berkeley, Berkeley, California
Dr. Robert T. Drew, Pharmacology and Toxicology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina
Dr. Peter Goldman, Beth Israel Hospital, Boston, Massachusetts
Dr. Robert E. Greenfield, Deputy Program Director, National Bladder Cancer Project, St. Vincent Hospital, Worcester, Massachusetts
Dr. Gert L. Laqueur, Chief, Laboratory of Experimental Pathology, National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, Maryland
Dr. Hermann Lisco, Associate Professor of Anatomy, Department of Anatomy, Harvard Medical School, Boston, Massachusetts
Dr. Robert H. Palmer, Director, Center for Prevention of Premature Arteriosclerosis, The Rockefeller University, New York, New York

Carcinogenesis Program Scientific Review Committee B

Members: Dr. Marcia D. Litwack, Executive Secretary
Dr. James Flesher, Pharmacologist, College of Medicine, University of Kentucky, Lexington, Kentucky
Dr. Adele Kostellow, Associate Professor of Physiology, Albert Einstein, College of Medicine, New York, New York
Dr. Prabhakar D. Lotlikar, Senior Investigator and Associate Professor of Biochemistry, Fels Research Institute, Temple University, Philadelphia, Pennsylvania
Dr. Danuta Malejka-Giganti, Veterans Administration Hospital, Minneapolis, Minnesota
Dr. Paul Talalay, Professor and Director, Department of Pharmacology and Experimental Therapy, Johns Hopkins University, Baltimore, Maryland

Dr. Floie M. Vane, Senior Chemist, Hoffmann-La Roche Inc., Nutley, New Jersey
Dr. Nien-chu C. Yang, Professor, Department of Chemistry, University of Chicago, Illinois

Carcinogenesis Program Scientific Review Committee C

Members: Dr. James Sontag, Executive Secretary
Dr. Gerald L. Bartlett, Assistant Professor, Department of Pathology, Pennsylvania State University, College of Medicine, Hershey, Pennsylvania
Dr. Robert M. Dougherty, Professor of Microbiology, State University of New York, Upstate Medical Center, Syracuse, New York
Dr. Veronica M. Maher, Chief, Carcinogenesis Laboratory, Michigan Cancer Foundation, Meyer Prentis Cancer Center, Detroit, Michigan
Dr. Roy Ritts, Chairman, Department of Microbiology, Mayo Graduate School of Medicine, Rochester, Minnesota
Dr. Andrew Sivak, Arthur D. Little, Inc. Cambridge, Massachusetts
Dr. Paul Ts'o, Professor and Director, Division of Biophysics, Department of Biophysics and Biophysical Sciences, Johns Hopkins University, Baltimore, Maryland
Dr. Willie Turner, Chairman, Department of Microbiology, Howard University, College of Medicine, Washington, D.C.

(4) A number of distinguished scientists have provided overall discussion and advice to the Carcinogenesis Program staff on the development of the program and its priorities in the context of future activities of the National Cancer Institute. Their individual activity replaced the previous Carcinogenesis Advisory Panel.

Advisors: Dr. John Baldeschwieler, Chairman, Department of Chemistry, California Institute of Technology, Pasadena, California
Dr. John Burns, Vice President for Research, Hoffmann-La Roche, Inc. Nutley, New Jersey
Dr. Emmanuel Farber, Professor of Pathology, Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania
Dr. Theodore Hauschka, Director, Cancer Research (Biology) Roswell Park Memorial Institute, Buffalo, New York
Dr. Ruth Kirchstein, Director, National Institute of General Medical Sciences, Bethesda, Maryland
Dr. Paul Kotin, Vice President, Health, Safety and Environment, Johns-Manville, Denver, Colorado
Dr. Peter Magee, Professor of Biochemistry, Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, England
Dr. James A. Miller, Professor of Oncology, McArdle Memorial Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

Dr. Norton Nelson, Director, Institute of Environmental Medicine, New York University Medical Center, New York, New York
Dr. Jesse Steinfeld, University of California, Irvine, California
Dr. Arthur Upton, Dean, School of Basic Health Sciences, State University of New York, Stony Brook, New York
Dr. Gerald Wogan, Professor of Food Toxicology, Massachusetts Institute of Technology, Cambridge, Massachusetts

Close liaison has been established with the National Cancer Advisory Board and its Subcommittee on Environmental Carcinogenesis.

Other consultants who have been utilized by the Program throughout this fiscal year as contract site visitors, reviewers, individual technical advisors, or lecturers are as follows:

Dr. J. Adams, Pharmaceutical Manufacturers Association
Dr. F. Aldrich, Massachusetts Institute of Technology
Dr. H.A. Anderson, Mount Sinai School of Medicine
Dr. E. Andrews, Cornell University
Dr. V.E. Archer, NIOSH
Dr. L. Aurelian, Johns Hopkins University School of Medicine
Dr. P. Bannasch, Institut Fur Experimental Pathologie, Heidelberg, West Germant
Dr. F.C. Bartter, NHLI
Dr. D.L. Bayliss, NIOSH
Dr. F.F. Becker, N.Y. University Medical Center
Dr. J.R. Bend, NIEHS
Dr. J.W. Berg, University of Iowa Medical School
Dr. E. Bingham, Kettering Laboratory, University of Cincinnati School of Medicine
Dr. H.P. Blejer, NIOSH
Dr. D.P. Boyd, OSHA
Dr. R.K. Boutwell, McArdle Laboratory for Cancer Research, University of Wisconsin
Dr. L. Brandborg, V.A. Hospital at San Francisco
Dr. J. Braun, NIEHS
Dr. T. Briggs, University of Oklahoma
Dr. P. Brookes, Chester Beatty Research Institute, Buckinghamshire, England
Dr. B.B. Brown, S.B. Penick and Company
Dr. D.B. Brown, Carnegie Institution of Washington
Dr. V. Brum, V.A. Center at Togus
Dr. H. Burchfield, Gulf South Research Institute
Dr. B.W. Carnow, University of Illinois School of Public Health
Dr. S. Carson, Biometric Testing, Inc.
Dr. B.Chance, Johnson Research Foundation, University of Pennsylvania
Dr. A. Chiarodo, NCI
Dr. L. Chiazze, Georgetown University School of Medicine
Dr. H. Christensen, NIOSH
Dr. E. Chu, University of Michigan School of Medicine
Dr. J. Churg, Mount Sinai School of Medicine
Dr. J.D. Clark, University of Georgia College of Veterinary Medicine
Dr. J.E. Cleaver, University of California Medical Center
Dr. N.H. Colburn, University of Michigan Medical School
Dr. J.S. Cook, Oak Ridge National Laboratory
Dr. W.C. Cooper, Tabershaw-Cooper Associates, Inc.
Dr. M. Copeland, M.D. Anderson Hospital and Tumor Institute
Dr. T. Corbett, University of Michigan Medical Center

Dr. F. Coulston, Albany Medical College
Dr. W. D'Aguanno, FDA
Dr. L.B. Dale, EPA
Dr. T.L. Dao, Roswell Park Memorial Institute
Dr. A.K. Davis, NCTR
Dr. P. Decoufle, NIOSH
Dr. K. Elgjo, Institutt for Patologi, Universitetet i Oslo, Oslo, Norway
Dr. A. Englund, Landsorganisationen i Sverije, Stockholm, Sweden
Dr. S.S. Epstein, Case Western Reserve University Medical School
Dr. B. Ewald, Cornell Medical College
Dr. W. Eyestone, University of Missouri School of Veterinary Medicine
Dr. H. Falk, Center for Disease Control
Dr. P. Feigelson, N.Y. Academy of Sciences
Dr. D.J. Fink, NCI
Dr. H.I. Firminger, University of Maryland School of Medicine
Dr. R.J. Flynn, Argonne National Laboratory
Dr. P.D. Forbes, Temple University Health Sciences Center
Dr. B. Fowler, NIEHS
Dr. A.J. Fox, Department of Employment, London, England
Dr. M.H. Friedman, Sloan-Kettering Institute of Cancer Research
Dr. J.J. Gart, NCI
Dr. P. Gehring, Dow Chemical Co.
Dr. J. Gilbert, Harvard Computing Center
Dr. J.D. Gillam, NIOSH
Dr. R. Gingell, Eppley Institue for Research in Cancer
Dr. M.C. Glick, Children's Hospital at Philadelphia
Dr. V.L. Go, Saint Mary's Hospital at Rochester
Dr. L. Golberg, The Albany Medical College of Union University
Dr. M. Gosalvez, Clinica Paerta de Hierro, Madrid, Spain
Dr. W. Gossner, Pathologisches Institut des Klinikums re. d. Isar, München, West Germany
Dr. S. Gouvain, Department of Employment, London, England
Dr. S.D. Greenberg, Baylor College of Medicin
Dr. Harold Grice, Food and Drug Directorate, Ottawa, Ontario, Canada
Dr. J.W. Grisham, University of North Carolina School of Medicine
Dr. D. Groth, NIOSH
Dr. E.C. Hammond, American Cancer Society
Dr. Carl Hansen, DRS
Dr. W. Harris, Uniroyal Company
Dr. R. Hart, Oak Ridge National Laboratory
Dr. L. Hayflick, Stanford University
Dr. C. Heidelberger, McArdle Laboratory, University of Wisconsin
Dr. E.G. High, McHarry Medical School
Dr. R. Hilf, University of Rochester School of Medicine and Dentistry
Mr. A. Hilgar, Saint Vincent Hospital at Worcester
Dr. T. Hirayma, National Cancer Center Research Institute, Tokyo, Japan
Dr. C.F. Hollander, Institute for Experimental Gerontology, Organization for Health Research TNO, Rijswijk, The Netherlands
Dr. R. Holley, Salk Institute for Biological Studies
Dr. L. Houten, Roswell Park Memorial Institute
Dr. P.F. Infante, Ohio Department of Health
Dr. N. Irey, Armed Forces Institute of Pathology
Dr. C. Irving, V.A. Hospital at Memphis
Dr. P. Issenberg, Eppley Institute for Research in Cancer
Dr. D. Janss, FCRC
Dr. B. Jaroslow, Argonne National
Dr. D. Joftes, NCI
Dr. K. Johnson, Manufacturing Chemists Association
Dr. W.M. Johnson, University of Arizona

Dr. D.C.L. Jones, Stanford Research Institute
Dr. J. Jones, NIOSH
Dr. E. Kaighn, W. Alton Jones Cell Center
Dr. Hans E. Kaiser, University of Maryland School of Medicine
Dr. R.E. Kallio, University of Illinois School of Life Sciences
Dr. R. Kato, Fujisawa Pharmaceutical Company, Ltd., Osaka, Japan
Dr. J.G. Keller, Mobil Oil Corporation
Dr. S.C. Kelton, Jr., Rohm and Haas Co.
Dr. M.M. Key, Potomac, Maryland
Dr. R.J. Kociba, Dow Chemical Company
Mr. J.C. Kolojeski, EPA
Mr. A.J. Konvicka, NCTR
Dr. K. Kraemer, University of Miami School of Medicine
Dr. C. Kuh, Washington University School of Medicine
Dr. N. Lane, Columbia University College of Physicians and Surgeons
Dr. I. Lasnitzki, Strangeways Research Laboratory, Cambridge, England
Dr. D. Lassiter, OSHA
Mr. L. Lawrence, NCTR
Dr. I. Leav, Angell Memorial Hospital
Dr. J.S. Lee, NIOSH
Dr. W.R. Lee, University of Manchester, Manchester, England
Dr. R.A. Lemen, NIOSH
Dr. W. Lijinsky, Oak Ridge National Laboratory
Dr. R. Love, Jefferson Medical College
Dr. F.E. Lundin, Bureau of Radiological Health
Dr. C.D. Lytle, FDA
Dr. P.N. Magee, Middlesex Hospital Medical School, London, England
Dr. C. Maltoni, Istituto di Oncologia "Felice Addarii", Bologna, Italy
Dr. T.F. Mancuso, University of Pittsburgh School of Public Health
Dr. S.J. Mann, Temple University
Dr. C. Marcelo, University of Michigan Medical School
Dr. D. Marlow, EPA
Dr. T.J. Mason, NCI
Dr. J. McCann, University of California at Berkeley
Dr. J.C. McEwan, Ministry of Health, Ontario, Canada
Dr. J. McLaughlin, Consumer Product Safety Commission
Dr. A.J. McMichael, University of North Carolina
Dr. B.P. McNamara, Edgewood Arsenal
Dr. M.L. Mendelsohn, Lawrence Livermore Laboratory, University of California
Dr. Calvin Menzie, Department of Interior
Dr. C.J. Michejda, University of Nebraska
Dr. S. Milham, Jr., Washington State Department of Health
Dr. J. Miller, McArdle Laboratory for Research on Cancer, University of Wisconsin
Prof. Dr. Med. Ulrich Mohr, Medizinische Hochschule Hannover, Hannover, West Germany
Dr. C.A. Moodie, Health and Welfare of Canada, Ottawa, Ontario, Canada
Dr. G.A. Morrill, Albert Einstein College of Medicine
Dr. E. Moss, University of Manchester, Manchester, England
Dr. F.K. Mostofi, Walter Reed Army Medical Center, AFIP
Dr. W.R. Muir, Council on Environmental Quality
Dr. J. Murdock, Battelle Columbus Laboratories
Dr. N. Nelson, New York University Medical Center
Dr. P.M. Newberne, Massachusetts Institute of Technology
Dr. M.L. Newhouse, London School of Hygiene and Tropical Medicine, London, England
Dr. V.A. Newill, Exxon Corporation
Dr. J.A. Newman, Butte Memorial Hospital
Dr. W.J. Nicholson, Mount Sinai School of Medicine of the City University of New York

Dr. J. O'Connor, Lehigh University
Dr. R.B. Painter, University of California School of Medicine
Dr. B. Papermaster, University of Texas Medical Center

Dr. S. Pell, E.I. Du Pont deNemours and Company
Dr. L. Plumlee, EPA
Dr. H. Popper, Mount Sinai School of Medicine of the City University of New York
Dr. J.D. Prejean, Southern Research Institute
Dr. E.P. Radford, Johns Hopkins University School of HYgiene and Public Health
Dr. D. Rall, NIEHS
Dr. R.E. Rasmussen, Cancer Research Institute, University of California School of Medicine
Dr. R. Rawson, M.D. Anderson Hospital and Tumor Institute
Dr. C. Redmond, University of Pittsburgh School of PUblic Health
Dr. D.J. Reed, Oregon State University
Dr. M.D. Reuber, Columbia, Maryland
Dr. H.L. Richardson, EPA
Dr. W.R. Richter, University of Chicago School of Medicine
Dr. N.J. Roberts, Exxon Corporation
Dr. A.E. Rogers, Massachusetts Institute of Technology
Dr. W. Rosse, Duke University School of Medicine
Dr. A. Rothstein, Hospital for Sick Children, Toronto, Ontario, Canada
Dr. V.K. Row, Dow Chemical Company
Dr. F.H. Ruddle, Yale University
Dr. H.P. Rusch, McArdle Laboratory for Cancer Research, University of Wisconsin
Dr. G. Saccommano, Saint Mary's Hospital at Grand Junction
Dr. H. Sakabe, National Institute of Industrial Health, Kawasaki, Japan
Mr. S. Samuels, AFL/CIO
Dr. E. Sawicki, EPA
Dr. D. Scarpelli, University of Kansas Medical Center
Dr. M.D. Scharff, Albert Einstein College of Medicine
Prof. A. Schauer, University of Göttingen, Göttingen, West Germany
Dr. M. Schulman, Energy Research and Development Administration
Dr. A. Schweid, Harborview Medical Center
Dr. A. Segaloff, Alton Ochsner Medical Foundation
Dr. I.J. Selikoff, Mount Sinai School of Medicine
Dr. B. Shaffer, American Cyanamid Company
Dr. R.E. Shapiro, FDA
Dr. R.E. Shenefelt, University of Arkansas Medical Center
Dr. S.I. Shibko, FDA
Dr. M.B. Shimkin, University of California School of Medicine
Dr. L. Siminovitch, Ontario Cancer Institute, Toronto, Ontario, Canada
Dr. R. Simpson, NIAMDD
Dr. M. B. Slomka, Shell Chemical Company
Dr. C.W. Smith, Shell Oil Company
Dr. E.R. Smith, Mason Research Institute
Dr. W.M. Smith, Air Products and Chemicals
Dr. E. Smuckler, University of Washington
Dr. K.C. Snell, Silver Spring, Maryland
Dr. S. Sorokin, Harvard University School of Public Health
Dr. A. Sparks, National Oceanic and Atmospheric Administration
Dr. J. Stillman, Oil Chemistry and Atomic Workers Union
Dr. E.L.R. Stokstad, University of California
Dr. D. Stoltz, Food and Drug Directorate, Ottawa, Canada
Dr. E.E. Storrs, Gulf South Research Institute
Dr. J.D. Strandber, Johns Hopkins University School of Medicine
Dr. B. Strauss, University of Chicago
Dr. O. Stutman, Sloan-Kettering Institute
Dr. W. Summers, Yale University Medical School
Dr. H. Swann, Downey, California
Dr. H. Swift, University of Chicago
Dr. R.G. Tardiff, EPA
Dr. F. Tate, Chemical Abstracts Service, Ohio State University

Dr. A.S. Tegeris, Pharmacopathics Research Laboratories, Inc.
Dr. L. Tomatis, International Agency for Research on Cancer, World Health Organization, Lyon, France
Dr. T.R. Torkelson, Dow Chemical Co.
Dr. A.C. Upton, Health Sciences Center, State University of New York
Dr. H.M. Vars, University of Pennsylvania School of Medicine
Dr. E.C. Vigliani, University of Milan, Milan, Italy
Dr. J.J. Vitale, Mallory Institute of Pathology
Dr. R.W. Voelker, Hazleton Laboratories
Dr. R.C. Wands, National Research Council, Assembly of Life Sciences
Dr. R.J. Wzxweiler, NIOSH
Dr. P.D. Webster II, V.A. Hospital at Augusta
Dr. W. Wechsler, Max-Planck Institut für Hirnforschung, Cologne, Germany
Dr. C.S. Weil, Carnegie Mellon Institute of Research
Dr. I.B. Weinstein, College of Physicians and Surgeons at Columbia University
Dr. C.J. Wessel, Tracor Jitco, Inc.
Dr. I.J. Wilk, Menlo Park, California
Dr. G. Williams, Fels Research Institute, Temple University School of Medicine
Dr. D.A. Willigan, Donald A. Willigan, Inc.
Dr. B.J. Wilson, Vanderbilt University School of Medicine
Dr. G.P. Wogan, Massachusetts Institute of Technology
Dr. S. Wolfe, Health Research Group
Dr. E Wynder, American Health Foundation, Inc.
Dr. J.A. Zapp, Jr., E.I. Du Pont de Nemours, Inc.
Dr. C. Zurcher, Institute for Experimental Gerontology TNO, Rijswijk, The Netherlands
Dr. G. Zweig, EPA

4. SCIENTIFIC ACTIVITIES; PROGRESS HIGHLIGHTS

The Carcinogenesis Program has continued to expand and to cover a broad front in its attack on the problem of identification of causative factors and the prevention of cancers. Many of the individual projects in this coordinated program have been underway for several years and represent a patient, intensive, and costly long-term effort towards the attainment of our specific program goals. Work in chemical carcinogenesis is characterized to a large extent by the multi-year duration of lifespan studies in laboratory animals, with a resultant long-range commitment of efforts and resources. Other projects represent a complex set of interrelated activities targeted to develop and refine the research methods necessary for study of the chemical, biochemical, biological, and molecular mechanisms that are responsible for the host response to chemical and physical carcinogens, and for the identification of exploitable preventive measures.

The Program's scientific accomplishments derive both from the intramural laboratories and from contract-supported projects: these cover fundamental research on the basic mechanisms of carcinogenesis, model and method developments, cancer pathogenesis studies, identification of carcinogens, and development of resources. Summary reports are provided by the branches and segments.

Highlights are summarized here in the perspective of the overall activity of the Program.

A. TECHNICAL INFORMATION AND PROGRAM RESOURCES

The provisions of the National Cancer Act of 1971 and of the National Cancer Plan emphasize the need for an expanded, intensified, and coordinated research program encompassing the programs of the National Cancer Institute, related programs of other research institutes, and other Federal and non-federal programs as well as the need to collect, analyze, and disseminate data useful in the prevention, diagnosis, and treatment of cancer.

Although the responsibility for specific regulatory activities and decisions (which are intended to accomplish the removal or minimization of exposure as well as to provide safeguards against exposure of the population to carcinogenic chemicals) is maintained by the present regulatory agencies--such as the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), the Department of Labor's Occupational Safety and Health Administration (OSHA)--the NCI's Carcinogenesis Program has provided an essential advisory function and has played an increasing role in the development of documentation and the elaboration of regulatory criteria in the Federal Government related to environmental carcinogens.

In this capacity, the Program has developed close liaison with other agencies in DHEW, as well as other departments, agencies, and programs in the Federal Government and has participated in many review and policy meetings at their request. The following activities are especially noteworthy:

- Staff has participated in the DHEW Committee to Coordinate Toxicology and Related Programs and its subcommittees: one dealing with the drafting of department standards on the use of chemical carcinogens in the laboratory; one on proposed guidelines as to how mathematical and statistical procedures might be applied to laboratory animal data in the extrapolation to low levels and used to estimate possible risks for human populations; one on toxicology information to collect, store, and disseminate appropriate data and information with respect to toxicologic and related activities; and one on the evaluation of toxicity data on nitrilotriacetic acid (NTA).

- Data have been collected and analyzed, scientific evaluation has been provided and testimony prepared at the request of EPA for hearings on the pesticides, aldrin and dieldrin, mirex, and chlordane and heptochlor.

- Standards for occupational carcinogens have been reviewed for OSHA. Safety guidelines have been developed for use by laboratory workers in research involving chemical carcinogens in coordination with DHEW and the Department of Labor.

- Staff participated in a number of interagency groups including the following: Science Advisory Board and Policy Board of the National Center for Toxicological Research, FDA; Interdepartmental Study Group on Nitrosamines-Nitrites and Amines organized jointly by FDA and USDA; Interagency Panel on Environmental Mutagenesis, organized by the National Institute of Environmental Health Sciences (NIEHS); Meetings of the Office of Toxic Substances, EPA, to compile the report entitled "Activities of Federal Agencies Concerning Selected High Volume Chemicals"

- The Interagency Collaborative Group on Environmental Carcinogenesis, formerly sponsored by the Carcinogenesis Program and now by the Office of the Director, DCCP, provides a useful channel of communication to alert other agencies on cancer problems. In turn, the participating regulatory agencies have alerted this Program as to environmental concerns which require action in the areas of additional studies, expert evaluation, or information gathering.

- Assistance was provided to the National Institute of Occupational Safety and Health (NIOSH) on problems in occupational carcinogenesis and in the review of drafts of criteria and standards for occupational exposures.

- Assistance was provided to EPA in the review of pesticide evaluations, water pollutants, proposed effluent water standards, and standards for toxic pollutants.

International liaison and collaboration has been maintained through participation to committees and advisory groups of the following organizations:

- International Agency for Research on Cancer (IARC) of the World Health Organization

- The US-USSR program on tumor cell genetics. This program is concerned primarily with mammalian somatic cell genetics related to neoplasia. Two areas will be developed--mutagenesis and cell hybridization. Dr. J. A. DiPaolo of the Carcinogenesis staff is chairman of the US delegation.

- A panel on mutagenesis and carcinogenesis was established and held a meeting in conjunction with the NIEHS for the US-Japan Coooperative Medical Science Program.

The following workshops and conferences were organized and/or sponsored directly by the Carcinogenesis Program and/or in collaboration with other agencies:

- Workshop on Chemical Mutagenesis sponsored by the Carcinogen Metabolism and Toxicology Segment, held in Bethesda, Maryland, on June 17-19, 1974

- Symposium on Experimental Respiratory Carcinogenesis and Bioassays, sponsored by the Battelle Memorial Institute, held in Seattle, Washington, on June 23-26, 1974. The program of this symposium was planned with the cooperation of the Carcinogenesis Program, NCI, and the Division of Biomedical and Environmental Research, ERDA (formerly AEC)

- Prostate Carcinogenesis Workshop, sponsored by the Biological Models Segment, held in Denver, Colorado, on September 6, 1974

- Workshop on Physical and Chemical Carcinogenesis and the Immune System, sponsored by the Biology Branch and the Biology and Immunology Segment, held in Reston, Virginia, on September 23-24, 1974

- Workshop on Vitamin A and Cancer Prevention sponsored by the Lung Cancer Segment and Hoffmann-La Roche, Inc., held in Bethesda, Maryland, on November 21-22, 1974

- Planning Conference on Colon Cancer, sponsored by the Colon Cancer Segment, held in Silver Spring, Maryland, on December 5-6, 1974

- Rat Liver Tumor Workshop, sponsored by the Carcinogenesis Program, held in Silver Spring, Maryland, on December 11-13, 1974

- Workshop on In Vitro Carcinogenesis, sponsored by the Carcinogenesis Program, held in Bethesda, Maryland on January 7-8, 1975

.Workshop on Experimental Designs for Evaluation of Carcinogenicity of Pesticides, sponsored by the Carcinogenesis Program, held in Bethesda, Maryland, on January 29-30, 1975

.Third Annual Carcinogenesis Collaborative Conference held in Orlando, Florida, on February 2-5, 1975

·Conference on Occupational Carcinogenesis, organized by the New York Academy of Sciences, and sponsored by Group Health, Inc., the National Cancer Institute (Carcinogenesis Program), NIOSH, and the Society for Occupational and Environmental Health, held in New York, New York, on March 24-27, 1975

(1) Information Resources

(a) A major program has been established for the collection and analysis of information on those chemicals that have significant impact on man and his environment. This activity provides support to the National Cancer Program by identifying those chemicals with which humans come in contact and by making available information on their structure, production, distribution, use, and estimated levels of exposure. The project has already provided detailed documentation on some 4,000 chemicals. Additionally, this documentation is provided to the IARC for inclusion in the IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man (Contractor: Stanford Research Institute, NO1-CP-33285, Information and Resources Segment).

This project will be of assistance to the whole Program in the following ways: by helping to choose those compounds having highest priority for entry into the bioassay testing program; by identifying trends in the exposure of man to chemicals which will anticipate problem areas and acquire relevant data; by identifying specific subpopulations which may be at high risk due to chemical exposures; and by identifying points at which such information may be interfaced with the activities of other agencies and bureaus, both nationally and internationally. Formal relationships have already been established between this project and groups outside of NCI (EPA, IARC, and the National Library of Medicine). These groups provide some degree of financial support as well as a continuing scientific input into the activities carried out under this contract.

(b) The series of IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man have been developed and published by the IARC with collaboration from the Carcinogenesis Program. So far seven volumes have been published and one more is in preparation covering a total of over 200 substances for which there is significant human exposure and some published evidence of carcinogenicity in animals and/or man. The chemicals reviewed in these monographs are grouped as follows: miscellaneous compounds (volume 1); some inorganic and organometallic compounds (volume 2); certain polycyclic aromatic hydrocarbons and heterocyclic compounds (volume 3); some aromatic amines, hydrazine and related substances, N-nitroso compounds and miscellaneous alkylating agents (volume 4); some organochlorine

pesticides (volume 5); sex hormones (volume 6); some anti-thyroid and related substances, nitrofurans and industrial chemicals (volume 6); and aromatic azo dyes (volume 8 - in press) (Contractor: IARC, NO1-CP-45608, Information and Resources Segment).

The increasing number and quantity of chemicals introduced into the environment and their possible adverse effect, especially with regard to carcinogenicity call for a coordinated effort to assess the magnitude of the resulting risk so that it is possible for preventive measures to be taken. Only an international organization, such as IARC/WHO could convene the expert committees essential for the critical review of published data on the carcinogenic activity of selected compounds. These monographs constitute the most authoritative source available on carcinogenicity evaluations, widely distributed to governments and research agencies. As a result of the documentation on the carcinogenicity of a large number of chemicals currently in use, recommendations have been forwarded to DHEW and particularly NIOSH, as well as OSHA, for occupational monitoring of people exposed to such agents.

(c) The collection, publication, and dissemination of information from the published literature on carcinogenesis studies (Carcinogenesis Abstracts) and on carcinogenesis bioassays (Survey of Compounds Which Have Been Tested for Carcinogenic Activity) has been continued by the Carcinogenesis Program.

The journal, Carcinogenesis Abstracts, is an up-to-date abstract service distributed to carcinogenesis researchers throughout the world in a format designed to provide relevant information on the various disciplines which relate to cancer etiology. Two new indexes are being added to increase the effectiveness and utility of this publication (Chemical Abstracts Services Registry number and Wiswesser line notation of the chemicals). Abstracts of current and previous volumes are being prepared for entry into Toxline, a National Library of Medicine's on-line Bibliographic Search System. The subset of Toxline containing Carcinogenesis Abstracts and Cancer Therapy Abstracts is entitled "Cancerline" (Contractor: Franklin Institute Research Laboratories, NO1-CP-43293, Information and Resources Segment).

Public Health Service Publication No. 149 series entitled Survey of Compounds Which Have Been Tested for Carcinogenic Activity has been brought up to date. The 1970-1971 volume has been published and the 1972-1973 volume is in preparation (Contractor: Tracor Jitco, Inc., NO1-CP-33402, Information and Resources Segment). In addition, the IARC, in an attempt to reduce duplication in testing efforts has initiated an international registry of compounds undergoing long-term carcinogenesis testing and has published an Information Bulletin on Chemicals Being Tested for Carcinogenicity.

These publications provide useful tools to identify those chemicals which have been tested for carcinogenicity (as reported in the literature) and the adequacy of such tests and also provide some information on substances being tested in other laboratories throughout the world.

Publications related specifically to the bioassay operations program will be reported under Section 4, B, (1).

(d) Publications of research results is of vital importance to the overall National Cancer Program. A comprehensive listing of publications arising from both intramural and collaborative activities is included in this report under Section 7. The total number of publications in FY 1975 is as follows:

Program	No.	Percentage
Intramural	182	30.5%
Collaborative	376	63.1%
Joint*	38	6.4%
TOTAL	596	100.0%

*Co-authorship by staff from intramural and collaborative programs.

(2) Animal Resources

Facilities to help meet our needs in the area of animal resources have been expanded through the acquisition of a large animal farm at the NCI's Frederick Cancer Research Center (FCRC). This source is now capable of providing well-characterized, uniform, and healthy animals for a large proportion of our bioassay program (Contractor: NCI/FCRC [Litton Bionetics, Inc.], NO1-CO-25423 [Project 12], Information and Resources Segment).

Provisions for making available adequate supplies of healthy, genetically uniform and well-characterized animals is essential to the continued conduct of the Carcinogenesis Program. In addition, it is necessary to provide outbred stocks and an increased number of experimental animals which will serve as models for particular human diseases.

The Carcinogenesis Program utilizes large numbers of laboratory animals in a wide variety of program efforts, both intramurally and collaboratively. The mice, rats, hamsters, and guinea pigs are obtained principally from the animal farm of the NCI's Frederick Cancer Research Center, but additional animals are received from the Veterinary Resources Branch, NIH and from the contract procurement activity of the Division of Cancer Treatment, NCI. The programs at the ERDA's Oak Ridge National Laboratory (YO1-CP-50200) and the Eppley Institute for Research on Cancer (University of Nebraska, NO1-CP-33278) are using mostly animals from their in-house breeding colonies. The estimated total of all animals allocated for FY 1975 in the Carcinogenesis Program is expected to be about 210,000. In addition it is estimated that approximately another 110,000 animals are being maintained in the course of long-term studies.

Additional activities concern the development of new or improved animal resources for carcinogenesis research. One such effort has resulted in the development and characterization of the wild European hamster (Cricetus cricetus) as a valuable experimental animal for carcinogenesis studies. Studies with several carcinogens have confirmed the susceptibility of this species to epidermoid lung cancer of a type similar to that seen in man. In addition, an excellent, well-illustrated anatomical atlas of this animal has been produced and is now being reviewed (Contractor: Medizinische Hochschule Hannover, NO1-CP-12148, Information and Resources Segment). A breeding colony has been established in the United States.

It is planned to continue the activities underway and to expand the program into areas where specific resource needs are identified which will facilitate program development.

(3) Chemical Resources

Resource activities have been developed which are designed to meet program needs in terms of acquisition, synthesis, characterization, and distribution of chemicals used for bioassay testing or as reference standards. This activity is of sufficient magnitude that a centralized repository is being planned which will assure that such materials are handled, packaged, and shipped under conditions consistent with the protection of laboratory personnel and the general environment from contamination with carcinogenic materials.

Specific collaborative efforts in this area include the following: (a) a carcinogen reference bank presently containing over 70 well-characterized reference compounds is in operation at its temporary location (Contractor: NCI/FCRC [Litton Bionetics, Inc.], NO1-CO-25423 [Project 9], Information and Resources Segment); (b) a resource for the analysis and characterization of substances used in the conduct of studies in the total Carcinogenesis Program (Contractor: Midwest Research Institute, NO1-CP-23270, Information and Resources Segment); (c) preparation of particulate carriers for respiratory carcinogenesis studies having well-defined physical and chemical characterizatics (Contractor: IIT Research Institute, NO1-CP-33296, Information and Resources Segment); (d) synthesis of purine and pyrimidine nucleotides specifically tailored to function as selective irreversible inhibitors of nuclear enzymes (Contractor: Ash Stevens, Inc., NO1-CP-23293, Information and Resources Segment); and (e) synthesis of isotopically labelled N-nitroso compounds (University of New Hampshire, NO1-CP-55675, Research Triangle Institute, NO1-CP-55671, and Stanford Research Institute, NO1-CP-55672, Information and Resources Segment).

A project has been established to define methodology for the safe handling and disposal of carcinogenic chemicals, as a focal point in our Program for the development of standards of safe practices which would result in the protection of research workers and the surrounding community from exposure to chemical carcinogens (Contractor: NCI/FCRC [Litton Bionetics, Inc.], NO1-CO-25423 [Project 15], Information and Resources Segment). An

experienced chemist has been added to the Program staff to coordinate this important new area.

These chemical resources, which are available to investigators in the collaborative program as well as interested NCI investigators, provide a constant and dependable source of material for biological studies and ensure standard uniform materials for related experiments.

B. BIOASSAYS FOR CARCINOGENIC ACTIVITY

An effort is presently underway to submit a large number of chemicals to carcinogenicity bioassays in well-defined and standardized experimental conditions. Approximately 540 chemicals are being screened for carcinogenicity under contracts from the Bioassay Operations Segment. The animal population used this year for bioassay operations was approximately 225,000 animals. These include about 105,000 rats of the Osborne-Mendel, Fischer/344, and Sprague-Dawley strains; 105,000 mice of the Swiss, C57Bl/6 and hybrid B6C3F1 strains; and 15,000 animals of other species, mostly hamsters. Most chemicals are tested by a protocol that includes preliminary toxicity studies followed by life-time feeding tests on groups of 50 animals each in two species, both sexes, and at two dose levels (including a dose close to the maximum tolerated dose).

Guidelines have been established for standardized, detailed protocols for the major bioassay procedures and a more detailed pathology procedure has been established. These procedures will soon be published. With these developments and those for data acquisition and analyses, the Program is making an important transition. Until recently, the activity was primarily aimed at the screening of environmental chemicals to identify suspected carcinogens for further study. More attention is now being given to the generation of detailed data at a level of sophistication and control that is needed to assist other agencies who are responsible for the making of regulatory decisions.

The day-to-day management of an expanding program of this magnitude is an extremely demanding task. The demands placed on NCI personnel by this task were so extreme that, during last fiscal year, a prime contract was initiated for this purpose. This contract, with Tracor Jitco, Inc. (NO1-CP-43340, Bioassay Operations Segment), monitors all aspects of the bioassay program including experimental design, planning of resources (materials, animals, laboratory facilities), conduct of studies, data collection, and analysis and presentation of results. As existing contracts with commercial laboratories expired, the prime contractor negotiated and assumed responsibility for each of them as a subcontract. Major activities include the following: (1) conversion of ten former NCI bioassay contracts into subcontracts, with complete documentation of all scientific, management and cost aspects in case files; (2) performance of continued monitoring of the ten subcontracts; (3) establishment and

implementation of a management plan and system for the bioassay operations; (4) establishment and implementation of a laboratory resource qualification system, with staff visits to a total of 27 laboratories; (5) preparation and issuance of a competitive request for proposal for initiation of testing of additional chemicals; (6) acceleration of nomination, selection and approval as well as assumption of procurement activities for a series of chemicals to be tested; (7) establishment of an information system on all chemicals nominated; (8) coordination of an animal resource system; (9) commencement of preparative work on a series of technical reports on chemicals tested; and (10) development of improvements in quality of data and data input into the Carcinogenesis Bioassay Data System.

(1) Carcinogenesis Bioassay Data System (CBDS) and Technical Reports

Emphasis has been placed on the need for the collection of detailed information on all aspects of a carcinogenesis bioassay. A system has been designed and implemented by intramural staff to accomplish this collection as well as the related storage and retrieval function. The CBDS, fully operational since last year, receives data input on experimental design, clinical observations, survival, and pathology results from about 20 contractors. The routine data entry into CBDS is provided under contract (Contractor: Wolf Research and Development Corp., NOI-CP-43257, Information and Resources Segment).

Data collected from bioassay studies are important in formulating public policy pertaining to the use of the agents tested. It is necessary that the results of these studies be properly recorded and analyzed so that they may be effectively used in the decision-making process.

The system will be extended to facilitate data analysis using the best available techniques.

One of the most pressing concerns in the bioassay program is a need to publish a full documentation of each bioassay with all the details of the methodology, conduct of the test and results. While usual "short" reports in open literature or conferences will still be encouraged, a new Carcinogenesis Technical Report Series has now been established to provide for complete reporting of detailed carcinogenesis bioassay results. The first volumes are in preparation.

(2) Chemicals under Test

Approximately 540 chemicals, representing different categories of potential human exposure, are under test. Included are 112 pharmaceuticals, 82 industrial chemicals, 94 pesticides and agricultural chemicals, 36 metallic compounds, and in addition natural materials, food additives, and a variety of other environmental chemicals and intermediates.

The large expansion in the number of chemicals placed on test in FY 1972-1973 is now generating a large workload in pathology evaluation, data analysis and reporting. Approximately one-half of the chemicals on test are now in these last three phases. During the past year contract

scientists have reported carcinogenic activity for approximately 86 chemicals. These are listed in the Bioassay Operations Segment Summary Report. Among those found to be carcinogenic are a number of important industrial and environmental chemicals, as well as pharmaceuticals and pesticides currently undergoing review by other Federal agencies.

The wide distribution of pesticide chemicals in the environment makes it particularly important to identify members of this group which represent potential long-term hazards to man. The carcinogenicity of DDT, mirex, and aldrin and dieldrin has been conclusively established after extensive review of research results obtained by NCI, IARC/WHO, and industry studies. The NCI studies are generating additional data which should prove useful in formulating national policies on the problem of pesticide control.

It is planned to continue the studies underway and to identify additional chemical compounds which should be tested. Final evaluation of data from completed studies is proceeding as rapidly as personnel limitations permit. This activity is considered to be of highest priority.

In FY 1975 there was a period of reduction in the introduction of new chemicals into the bioassay program, due to the staffing, the large backlogs in pathology and reporting, the need for developing additional resources, and the work required for the initiation of the prime contractor. With the establishment of the prime contract activities, the program has now developed the capabilities to conduct carcinogenesis bioassays in an orderly, steady, and well-controlled manner. The testing activities, contributed from the laboratories coordinated by the prime contractor, as well as from research institutes and university laboratories, provide not only much needed new information on the carcinogenic potential of chemicals to which the population is exposed, but also greatly contribute to the development and evaluation of research methods and of pathology criteria relevant to the whole field of studies on carcinogenesis and cancer prevention.

(3) Selection of Chemicals for Bioassay

A new procedure has been developed for the process of priority selection of chemicals to be entered into the bioassay program. A complete long-term bioassay on two species, at two doses (on a total of approximately 500 animals), and with preliminary toxicological and final pathological evaluation is estimated to cost approximately $100,000. Therefore, an adequate review process for the commitment of resources for the bioassay of given chemicals was required. With the assistance of the prime contractor, a Chemical Selection Working Group has been activated with ad hoc expertise from outside consultants. This Working Group reviews extensive documentation of each chemical considered for bioassay and transmits its recommendations to an NCI staff committee (Chemical Selection Committee) for final action.

Nominations for chemicals to be considered for bioassay are received from a variety of sources, including other Federal agencies. See Bioassay Operations Segment Summary Report.

C. EXPERIMENTAL PATHOLOGY STUDIES OF CANCER INDUCTION AND PREVENTION

Studies designed to develop and define animal models of the principal types of cancer and to define their pathogenesis are a major component of the Carcinogenesis Program.

(1) Tumor Pathology

A reference center for animal tumor pathology has been established. The Tumor Pathology Section, which includes the Registry of Experimental Cancers, has considerably expanded this activity. Pathology protocols for bioassays have been developed in consultation with several leading pathologists. The Registry of Experimental Cancers has reached a total of 21,500 records of experimental tumors and functions as a national and international consultation center. Selected pathology studies of induced tumors have been carried out through the Program.

At the workshop on the interpretation of rat liver tumors, 20 participants reached a consensus on a classification and nomenclature for several controversial liver lesions which are frequently encountered in carcinogenesis studies. Of greatest significance was the recommendation to replace the widely used term "hyperplastic nodule" with "neoplastic nodule" since this lesion was considered a true neoplasm, and a stage in the development of hepatocellular carcinoma.

A major part of the data representing the results of carcinogenesis tests consists of a comparative pathological evaluation of lesions observed in test and control animals. Therefore, the Program needs comprehensive and detailed reference standards for the classification of spontaneous and induced tumors in common laboratory animals and their comparison with the corresponding tumors in man. The level of quality control imposed by this activity on the evaluation of endpoints in bioassay studies is critical to the success and acceptance of our screening activities.

The availability of qualified pathologists and of funds to support extensive pathology study of animals under test may prove to be the limiting factors that will determine the extent of the bioassay program. Two contractors (Contractors: Experimental Pathology Laboratories, NO1-CP-43300, and Johns Hopkins University, NO1-CP-43288, Information and Resources Segment) have provided pathology support to the Program, thereby improving program resources for both bioassays and experimental pathology.

(2) Lung Cancer

Respiratory tract carcinogenesis studies have made considerable progress, including studies on high risk human groups, experimental animal bioassay and pathogenesis studies, organ culture studies both in animal and on human tissues, and morphological-biochemical correlation studies on mechanisms of carcinogenesis and their selective inhibition by anticarcinogens.

Cytology studies are used to define the stages of development of bronchogenic carcinoma. Staff have participated in the establishment and follow up of a study of a high-risk population of workers formerly employed under heavy asbestos exposure conditions. This study is funded by the Division of Cancer Control and Rehabiliation, NCI, and by NIOSH under contract with the Texas Chest Foundation (Lung Cancer Branch).

A large group of uranium miners is being followed with sputum cytology in order to achieve a more accurate definition of the time and stage of development of lung cancer in this population of workers who are at such high risk for lung cancer. A method for respiratory cytology has been developed for use in laboratory animals, using a cannula that allows washing of a limited segment of the tracheobronchial tract, and a good correlation has been found between animals and man, in terms of the cytologic stages that occur during the development of bronchogenic squamous cell cancer. These studies suggest that preinvasive carcinoma can be detected cytologically and that accurate diagnosis of early invasive carcinoma is feasible. This finding is of great importance with respect to the problem of early diagnosis of human lung cancer and for monitoring the effectiveness of inhibitory treatments (Contractors: ERDA-Oak Ridge National Laboratory, YO1-CP-50200, Office of the Associate Director for Carcinogenesis and St. Mary's Hospital, NO1-CP-23233, Lung Cancer Segment).

A new method for the induction of respiratory tract tumors in hamsters has been developed, by delivering carcinogens through the cannula used for washing a well-defined and predetermined segment of the trachea. This new system allows tumor induction at a give site and will considerably facilitate detailed morphogenesis studies. This method has already been used in a study which for the first time correlates the histology of a given lesion with its own exfoliated cells (Contractor: ERDA-Oak Ridge National Laboratory, YO1-CP-50200, Office of the Associate Director for Carcinogenesis).

Respiratory carcinogenesis bioassay experiments have continued on several chemicals of environmental significance, by various techniques including inhalation and intratracheal instillation. Preliminary results indicate that nitrogen dioxide inhalation enhances the carcinogenic response to benzo[a]pyrene; a co-carcinogenic effect was also reported for croton oil inhalation. The carcinogens 7-H-dibenz[c,g]carbazole and dibenz[a,i]pyrene were found very active by intratracheal administration in hamsters without ferric oxide carrier particles, which are required for the effect of benzo[a]pyrene; this difference does not appear related to retention rate.

Synergistic effects have been shown in hamsters in the induction of lung cancer between alpha radiation from Polonium-210 and the chemical carcinogen benzo[a]pyrene (Contractor: Harvard University School of Public Health, NO1-CP-33273, Lung Cancer Segment) and between different sets of chemical carcinogens (Lung Cancer Branch). The European hamster was found to be susceptible to the chemical induction of bronchogenic carcinoma and provides an additional, larger animal model (Contractor: Medizinische Hochschule, Hannover, NO1-CP-12148, Information and Resources Segment).

The inhibition of carcinogenesis in the respiratory tract by vitamin A has been confirmed in a study in rats exposed to methylcholanthrene. These studies also indicate that rats maintained on low vitamin A intake have an increased susceptibility to methylcholanthrene-induced lung cancer (Contractor: ERDA-Oak Ridge National Laboratory, YO1-CP-50200, Office of the Associate Director for Carcinogenesis).

In another long-term study, 13-cis-retinoic acid, a less toxic synthetic analog of vitamin A, has been shown to prevent the development of lung cancer in animals that have previously been treated with benzo[a]pyrene (Contractor: IIT Research Institute, NO1-CP-23292, Lung Cancer Segment).

Administration of vitamin A acetate, given during and after the period of carcinogen dosing, was shown to produce marked inhibition of the combined carcinogenic effects of benzo[a]pyrene and nitrosomethylurea on the respiratory epithelium of the hamster (Lung Cancer Branch).

Short-term and long-term organ culture methods developed in the laboratories of the Lung Cancer Branch were used to study the major morphologic and biochemical events in target cells in respiratory tract carcinogenesis. Higher binding of benzo[a]pyrene to DNA was found in hamster strains in higher susceptibility to its carcinogenic action and also in vitamin A-deficient animals. Conversely, inclusion of high levels of retinol acetate in the organ culture medium inhibits binding of benzo[a]pyrene to DNA. These studies are pertinent to questions regarding genetic susceptibility and nutritional influence upon the development of lung cancer and on the possibility of modulating this development. Long-term organ culture systems have been well established and extensively used. Good survival of the organ cultures of tracheobronchial epithelium was found both for human and for rodent tissues. Human respiratory epithelium has been characterized by electron microscopic and cytochemical techniques, and has been placed in organ culture to study its response to carcinogens. Explants of human bronchi can be maintained for as long as six months in organ culture. These explants incorporate radioactive precursors into RNA, DNA and protein. Human bronchial epithelial cells in short-term organ culture have been found to metabolize several carcinogenic polynuclear hydrocarbons in forms that tightly bind to cellular macromolecules, including DNA. Binding occurs in both nucleus and cytoplasm of all major epithelial cell types and to their DNA, as shown by equilibrium density gradient banding in cesium chloride. Binding is decreased by 7,8-benzoflavone and butylated hydroxytoluene. Methods for xenotransplantation of human bronchus after maintenance in culture have been developed (Contractor: University of Maryland, NO1-CP-43237, Lung Cancer Segment).

Addition of asbestos to hamster tracheal organ cultures was found to cause early hyperplasia of respiratory epithelium. This hyperplasia may contribute to the co-carcinogenic or promoting effects of asbestos with respect to the problem of lung cancer (Lung Cancer Branch).

Continuous exposure of rat tracheal epithelium in organ culture to benzo[a]-pyrene at a concentration of 2 μg/ml for two weeks results in focal

proliferative response which persists for at least a month after removal of the carcinogen. This may be a premalignant change analogous to nodular hyperplasias in livers of animals discontinuously ingesting hepato-carcinogens (State University of New York [at Stony Brook], NO1-CP-33361, Lung Cancer Segment).

Other investigations include kinetic studies of the different cellular populations in respiratory epithelium (Contractor: VA Hospital [Tampa, Florida], YO1-CP-55625, Lung Cancer Segment). The role of mixed function oxidases in the activation of carcinogens in the respiratory epithelium is under extensive study (Contractors: University of Minnesota, NO1-CP-33364, University of Stockholm, NO1-CP-33363, and University of Texas [Southwestern Medical School], NO1-CP-33362, Lung Cancer Segment).

Several new synthetic analogs of vitamin A are being tested for their ability to inhibit squamous cell differentiation: New synthetic analogs of vitamin A have been found to have potent activity in controlling differentiation of respiratory epithelium, reversing keratinizing squamous metasplastic lesions. Some of these analogs are substantially less toxic than natural forms of vitamin A: they include compounds with ring modifications and others with modifications of the polar terminal portion of the molecule. The identification of specific anticarcinogens of low toxicity is a promising aim of these studies, for which invaluable collaboration was provided by Hoffman-La Roche, Inc. Some of these compounds have been found to inhibit microsomal mixed function oxidases and may, therefore, relate to the control of carcinogen metabolism (Contractor: Southern Research Institute, NO1-CP-22064, Lung Cancer Segment).

The molecular mechanisms of action of vitamin A were further investigated. It was previously found that tracheal epithelium can synthesize a mannolipid from retinol and GDP-mannose. This mannolipid has the structure of a retinol-phosphate-mannose type of compound and has been shown to function as a carrier for mannose during formation of glycoproteins. Retinyl phosphate has now been chemically synthesized for the first time and found to be a good substrate for mannosyl-transferase in an in vitro system. When incubated in the presence of epithelial membranes from liver or intestines, this compound accepts monosaccharides which are destined for membrane assembly. Mouse skin epidermal monolayers cultured in the presence of vitamin A synthesize a high molecular weight glycopeptide which is found in normal cultures and which contains both fucose and glucosamine. Vitamin A deficiency decreases the incorporation of labeled fucose and glucosamine into a tracheal epithelial glycopeptide from rat tracheal epithelium. A comparison of the content of retinol and retinyl esters in bronchogenic squamous cell carcinoma and intestinal adenocarcinoma showed that the squamous cell carcinoma has no detectable retinol or retinyl esters, in contrast to the adenocarcinoma, which has as much retinol and esters as normal intestinal cells (Lung Cancer Branch).

The prevention of lung cancer in man can only in part be achieved by removal of causative factors in our society. It is essential, therefore, that a concerted effort be made to identify all possible targets for protective

or inhibitory corrective measures at the host level. Continuation and development of this program is planned.

(3) Colon Cancer

A program in this area was established in FY 1972 in close collaboration with the Field Studies and Statistics Program, DCCP, NCI, and with the National Large Bowel Cancer Project. Its focus is on the correlation of colon cancer morbidity in migrant populations, the pathology of these cancers, and environmental dietary factors. The role of bacterial flora in the metabolism of intestinal carcinogens and in the susceptibility of population groups is being studied.

One hypothesis on the cause of human large bowel cancer suggest that intestinal bacteria metabolize chemical precursors to produce the actual carcinogen. Studies are underway on the characteristics and metabolism of bacteria isolated from the large bowel of individuals belonging to groups having differing risk for the development of colon cancer, such as rural Japanese, Japanese-Hawaiians, native South Africans, and Americans. Preliminary studies have shown that microorganisms isolated from subjects on a mixed diet degraded both neutral and acid steroids to a greater extent than those obtained from vegetarians. This observation is being followed up in both a quantitative and qualitative sense. A significant difference was noted in the degradation of cholesterol in the intestinal tract of persons with a history of adenomatous polyps when compared with control groups. Approximately 60% of the polyp patients converted less than half of the cholesterol or coprostanol and coprostanone as compared to 25% of the normal group. The feces of the "low convertors" contained lower total amounts of both animal and plant neutral sterols than did those of the control groups. Preliminary data suggest the following general principles: Drastic changes in diet do not greatly affect the fecal flora. The colonic flora is similar to the fecal flora. Anger-stress situations apparently affect the composition of the flora more than any other single factor tested. A major source of nutrient for intestinal organisms appears to be intestinal mucin (Contractor: Virginia Polytechnic Institute and State University, NO1-CP-33334, Colon Cancer Segment).

Although fewer colon tumors appeared in germ-free rats treated with 1,2-dimethylhydrazine than in conventional rats, it was demonstrated that the colon carcinogens 1,2-dimethylhydrazine and azoxymethane are active in germ-free rats, suggesting that these chemicals are converted to the ultimate carcinogens by the colon mucosa without intervention of intestinal microflora. More colon cancers were induced by dimethyl-hydrazine in rats on high fat diets compared to low fat diets. Colon cancer, similar to that seen in man, was induced for the first time in guinea pigs by the intrarectal instillation of methylnitrosourea and of methyl-nitronitrosoguanidine. Endoscopic examination was adapted to the early detection of colon cancer in rodents without requiring serial killing, thus facilitating studies in colon cancer pathogenesis (Contractor: American Health Foundation, NO1-CP-43378, Colon Cancer Segment).

Transplantable colon carcinomas, established in the laboratories of the Carcinogen Metabolism and Toxicology Branch were used for biochemical studies.

Colon cancer is one of the leading causes of cancer mortality in man. Although new leads are now available for the development and expansion of this program, lack of staff has made such expansion essentially impossible: Efforts will be continued on a limited scale in collaboration with the Field Studies and Statistics Program, to define the relationships of diet, intestinal flora, and the incidence of multiple colonic polyps and cancer; liaison will be maintained with the National Large Bowel Cancer Project.

(4) Pancreas Cancer

Exceptional progress has been made in the development of experimental models for the pathogenesis of pancreatic carcinoma. A group of projects was started mostly in FY 1972, to develop a scientific methodology for the definition of the pathogenesis and causative factors of this major form of cancer, the study of which had been so far neglected (Contractors: Boston University, NO1-CP-23297, Case Western Reserve University, NO1-CP-23284, University of Chicago, NO1-CP-33376, Dartmouth College, NO1-CP-33378, University of Kansas, NO1-CP-23271, Memorial Hospital for Cancer and Allied Diseases, NO1-CP-43232, University of Tennessee, NO1-CP-33369, Wright State University, NO1-CP-43231, Biological Models Segment).

Pancreatic adenocarcinoma has been induced in 36% of strain 13 guinea pigs within 36 weeks of treatment with methylnitrosourea: this markedly reduces the latent period and increases the incidence from previously reported studies (University of Kansas, NOL-CP-23271). Pancreatic adenomas and carcinomas were induced in a very high proportion of rats by treatment with azaserine (Contractor: Dartmouth College, NO1-CP-33378). Two other models are in a promising stage of development.

In addition, another group of investigators, working on the biological effect of nitrosamines, have found that a very high incidence of pancreatic tumors can be obtained in hamsters by 2,2'-dihydroxy-di-n-propylnitrosamine (Contractor: University of Nebraska [Eppley Institute], NO1-CP-33278, Office of the Associate Director for Carcinogenesis). The tumors are mostly ductal pancreatic adenocarcinomas, closely resembling those occurring in man. As many as 80% of the treated hamsters can develop pancreatic cancer as early as 15 weeks after beginning of treatment.

Some specific biochemical changes for carcinogenesis of the exocrine pancreas have been already identified. Nitrosamide carcinogens bind to RNA and DNA in guinea pig pancreas and uptake of these carcinogens appears greater in the head than in the tail of this organ. Cell and organ culture methods for studies on pancreatic carcinogenesis have been successfully established and are now being used in the further investigation of carcinogenic processes of this organ site.

Cancer of the pancreas results in over 19,000 deaths per year in the United States and the incidence is rising. The results obtained thus far provide a solid basis for further studies on this major type of cancer, for which no experimental model had previously been established.

More extensive laboratory experimentation is needed on those carcinogens which appear to affect the pancreas, the mechanisms responsible for the localization of their effects on the target cells of the exocrine pancreas, the presence of enzymes in the pancreas which may metabolize carcinogens to their active forms, and those physiologic and pathologic conditions which affect the localization of carcinogens and their metabolizing enzymes in the pancreas. The knowledge obtained from such an effort should make a substantial contribution to our ability to identify high risk factors and susceptible individuals in the population and to prevent this increasingly important form of human cancer.

A session of the Third Annual Carcinogenesis Collaborative Conference was devoted to pancreas carcinogenesis; its proceedings are to be published in Cancer Research.

(5) Other Organ-Oriented Programs

In mammary carcinogenesis, an interesting new model for the induction in organ culture of alveolar nodular structures resembling precancerous nodules seen in vivo has been reported (Contractor: University of Nebraska [at Lincoln], NO1-CP-33289, Biological Models Segment). Both estrogen and progesterone receptors have been demonstrated in the mammary epithelial cells of rats which were in a stage of development when tumors can be induced. Furthermore, the cytoplasmic-nuclear transfer of receptor appears to operate in the mammary gland. The amount of dimethylbenz[a]-anthracene bound to mammary epithelial DNA varies as a function at the age of the animal. There is a good correlation between the amount of dimethylbenz[a]anthracene bound and the incidence of tumors induced (Contractor: NCI /FCRC [Litton Bionetics, Inc.], NO1-CO-25423 [Project 16], Office of the Associate Director for Carcinogenesis). The development of organ culture studies on mouse mammary hyperplastic alveolar nodules has given preliminary results suggesting the induction of preneoplastic lesions in culture by 7,12-dimethylbenz[a]anthracene under specific hormonal manipulations (Contractor: University of Nebraska [at Lincoln], NO1-CP-33289).

Considerable new work was also developed in the study of carcinogenesis of several other organs and tissues (prostate, kidney, bladder, liver, skin, and of transplacentally induced cancers, particularly of the nervous system). A study of the late effects of cancer therapy in children has defined the incidence of "spontaneous" second malignant neoplasms and of those second malignant neoplasms that are probably related to the treatment of the first malignancy by radiation and/or chemotherapy. It was found that patients receiving actinomycin D have a sevenfold decrease in the risk of developing radiation-therapy-associated second malignant neoplasms; the risk is least in patients receiving multiple courses of actinomycin D (Memorial Hospital for Cancer and Allied Diseases, NO1-CP-23286, Office of the Associate Director for Carcinogenesis).

D. IN VITRO MODELS FOR CHEMICAL CARCINOGENESIS

A great need has been demonstrated in the area of environmental carcinogenesis for rapid, inexpensive methodologies which would permit the screening of substances considered for inclusion in long-term in vivo bioassay studies. Several in vitro systems for cell transformation by chemicals have been reported in the literature. Individually or collectively, these systems may fulfill the need for an effective screening method for carcinogenesis. It is necessary now to establish the reproducibility of these methodologies and to validate them by challenging them with a large number of highly purified chemicals of known carcinogenic activity (both positive and negative) and different chemical structure. A coordinated program on in vitro carcinogenesis has been planned and implementation has been initiated through the establishment of several contracts and the announcement for Cancer Research Emphasis Grants.

Causation of a large proportion of cancers in man has been attributed to environmental factors. All causative agents of cancers in man now known are either chemical or physical agents. The identification of new environmental carcinogens is still a costly and lengthy procedure. However, meaningful in vitro models for neoplastic transformation of cells by chemicals allow us to screen environmental chemicals for carcinogenic potential in a matter of a few weeks instead of two to three years. At the same time, such models give us a tool for identifying the key molecular events in the process of cancer induction in well-defined cell populations.

Since this is one of the areas of high priority in the Carcinogenesis Program, standardization and validation of existing techniques and development of new ones will be actively pursued. Expansion of this effort is projected in the following years. It is hoped that within this decade a battery of short-term test systems will be developed and validated to the extent that--taken collectively--it can be at least as predictive of carcinogenicity in man as are the currently used long-term bioassays.

Close coordination is being developed with related programs in Japan and in European countries through joint meetings and exchange of data.

(1) Cell Transformation Studies

Considerable progress has been made in this area, which is becoming one of the most productive and exciting fields of development in carcinogenesis.

Transformation of hamster and guinea pig embryo cells in vitro by chemicals has been previously reported (Biology Branch). It has now been shown that embryonic cells from random-bred, germ-free and SPF rats can be similarly transformed by chemicals in vitro. Morphologic alterations in carcinogen-treated guinea pig cells appeared shortly after treatment, but transformation and subsequently the capacity of the transformed cells to produce tumors occurred only after an extended period of time. Cloned cell lines with

differing morphology, growth characteristics and tumorigenicity have been isolated from the same parent mass culture of guinea pig cells. Comparative studies of chromosomal changes indicated that neither in vitro transformation nor in vivo tumor induction is accompanied by a specific numerical or structural chromosomal aberration. The synergism of chemical and physical agents or chemical and viral agents or different chemicals in the induction of transformation in vitro has been further documented.

Syrian hamster cell strains can be transformed by ultraviolet irradiation (2537 Å). When cells seeded to form colonies were treated with UV light followed by benzo[a]pyrene or N-acetoxy-fluorenylacetamide, the change in frequency of transformation was not statistically significant. Pretreatment of cells with X-ray enhanced UV-associated transformation (Cytogenetics and Cytology Section, Biology Branch).

A standardized procedure has been established for hamster embryo cell in vitro carcinogenesis bioassay, using aliquot samples of primary cells stored in liquid nitrogen as the source of standard target and feeder cells. To date, approximately 40 chemicals have been assayed in this system, including carcinogenic and noncarcinogenic analogs of some direct alkylating agents, polynuclear hydrocarbons, nitrosamines and nitrosamides, aromatic amines, amino azo dyes, and a few miscellaneous compounds. Almost complete correlation as to their reported carcinogenic activity in animal systems was found. A few procarcinogens such as diethylnitrosamine, N-2-acetylaminofluorene, auramine, and urethan, which require metabolic activation, failed to induce transformed colonies (Contractor: NCI/FCRC [Litton Bionetics, Inc.], NO1-CO-25423 [Project 10], Office of the Associate Director for Carcinogenesis).

Synergism between radiation and chemical carcinogens has been studied in the 10T-1/2 mouse cell line system. Synergism between x-rays and benzo[a]pyrene has been shown in this system which depends upon the sequence and time interval between treatments (Contractor: Harvard University School of Public Health, NO1-CP-33273, Lung Cancer Segment).

Using cultures of rat liver cells, the first mutants of chemically transformed epithelial cells that are temperature sensitive in the expression of the tumor cell phenotypes have been isolated and characterized. It has been discovered that glucocorticoid hormones inhibit the plasminogen activator production in certain chemically-transformed cells. A preparative method for enucleating mammalian cells has been developed (Contractor: Columbia University, NO1-CP-23234, Office of the Associate Director for Carcinogenesis).

Mouse epidermal cells in tissue culture were transformed by chemical carcinogens. They have been shown to undergo altered differentiation in response to vitamin A derivatives. This method provides an integrated in vitro/in vivo experimental system for study of the genesis of squamous cell carcinoma (In Vitro Pathogenesis Section, Experimental Pathology Branch).

Experiments on the factors involved in the in vitro cell transformation of a strain of mouse cells by chemicals have indicated that one cell generation

was required for the fixation of transformation and more than three cell generations are required for the expression of the transformed state. Caffeine was found to inhibit and acriflavin to enhance transformation frequency in this mouse cell system. Furthermore, preliminary data indicate that under very specific conditions normal human cells can be morphologically transformed by 4-nitro-quinoline-1-oxide and N-methyl-N'-nitro-N-nitrosoguanidine (Nucleic Acids Section, Chemistry Branch).

Five different xeroderma pigmentosum variant fibroblast cell strains have been found to be only about 70% as efficient as normal cells in repairing UV-irradiated adenovirus 2 DNA when this virus is used in a host cell reactivation assay developed in this laboratory. Most other methods used to measure DNA repair in human cells were unable to yield data indicating any repair defect in these xeroderma pigmentosum variant cell strains, in contrast to cells from classical xeroderma pigmentosum patients (Nucleic Acids Section, Chemistry Branch).

Studies on the effect of several chemical carcinogens on normal human foreskin cells in vitro showed, in certain culture conditions, an extension of the lifespan growth characteristics, including the ability to grow in soft agar. These effects were enhanced by 17-β-estradiol or cortisone acetate (Ohio State University Research Foundation, NO1-CP-43276, Biology and Immunology Segment).

A number of advances have been made using in vitro cell transformation models in the study of carcinogen metabolism and activation, surface changes, somatic cell genetics, and biochemistry of transformed cells.

(2) Mutagenesis

A series of three contracts are underway in the Carcinogen Metabolism and Toxicology Segment to determine the feasibility of using mutagenicity prescreens for the detection of potential environmental mutagens. The mutagenesis test systems are challenged with a series of 103 chemicals which include procarcinogens, ultimate carcinogens, structurally related non-carcinogens, and a few non-carcinogenic mutagens. The following test systems are screened.

Microbial gene mutations in Ames' tester strain of Salmonella following exposure to the compounds both in vivo and in vitro; recombination in yeast D-3 and inhibition of growth of repair deficient E. coli (Contractors: Columbia University, NO1-CP-33395, Stanford Research Institute, NO1-CP-33394); DNA repair, or unscheduled DNA synthesis, in human fibroblasts, following exposure to the compounds in vitro (Contractor: Stanford Research Institute, NO1-CP-33394); drug resistance to methotrexate, cytosine arabinoside and thymidine by 1-5178 y mouse lymphoma cells treated with each of the compounds in vivo and in vitro (Contractor: Arthur D. Little, Inc., NO1-CP-33392). The basis for these studies is that the activated forms of chemical carcinogens generally appear to be strongly mutagenic. Preliminary results from bacterial mutagenesis systems appear to correlate well with carcinogenicity. Mammalian cell systems need further development and validation.

(3) Somatic Cell Genetics

Somatic cell mutations resulting from the exposure of mammalian cells to chemical carcinogens are also under investigation. This has resulted in viable mutants, expressed in some cases by temperature sensitivity (Contractor: University of Illinois, NO1-CP-23303, Chemistry and Molecular Carcinogenesis Segment). Much work on the specific molecular mutations in the plasma membranes (of these mutants) is in progress to define changes in respect to specific transport systems and to plasma membrane systems capable of synthesizing cyclic nucleotides. The detailed studies on plasma membrane mutations will provide information regarding changes in cellular physiology arising through specific chemical mutation (Contractors: New York University, NO1-CP-33279; Ontario Cancer Institute, NO1-CP-43331, Chemistry and Molecular Carcinogenesis Segment). In addition, studies begun in the last few months are focused on the induction of somatic cell mutations other than those associated with the plasma membrane which affect the overall physiology of the cell (University of California [at San Francisco], NO1-CP-43239, Yale University, NO1-CP-55673, Chemistry and Molecular Carcinogenesis Segment).

A new, highly sensitive system was developed to test with mammalian cells the mutagenicity of carcinogens that have to be metabolically activated. It was found that the ability of mammalian cells to activate carcinogenic hydrocarbons metabolically can be experimentally increased (Weizmann Institute of Science, NO1-CP-02217, Chemistry and Molecular Carcinogenesis Segment).

These studies will be continued in an effort to correlate mutations arising from exposure to chemical carcinogens with specific morphological and physiological alterations in somatic cells.

(4) Organ Culture Studies

Considerable effort has been devoted to the development of organ culture methods for the maintenance of target tissues and their selective manipulation and study. Organ culture methods for respiratory tract carcinogenesis studies were developed in the Lung Cancer Branch in recent years and are extensively used both for short-term morphological-biochemical definition of the response to carcinogens and for long-term observation of induced changes up to advanced preneoplastic lesions. These methods have now been extended to the culture and study of human bronchial tissue, thus providing a major bridge in the correlation from animal to man in carcinogenesis. Several laboratories are contributing to the rapid advance of organ culture studies on the respiratory tract mucosa (Lung Cancer Segment).

Recently, conditions have been defined for the organ culture of rat urinary bladder in a chemically-defined medium. Under the conditions developed, bladder epithelium, which normally shows a slow rate of turnover, is stimulated to undergo a wave of basal cell replication which peaks after two days in culture and then subsides. This phenomenon has been previously seen in tracheal cultures (Lung Cancer Branch).

Organ culture studies on mouse mammary hyperplastic nodules exposed in vitro to a carcinogen and selective hormonal treatments have suggested the induction of preneoplastic lesions (University of Nebraska [at Lincoln], NO1-CP-33289, Biological Models Segment).

Additional organ culture methods have been investigated for prostate, pancreas, and skin (Experimental Pathology Branch and Biological Models Segment).

E. IMMUNOLOGICAL STUDIES IN CARCINOGENESIS

Radioimmunoassays have been developed for several carcinogens and related compounds, which can precisely detect minute traces of cancer-causing chemicals in body fluids. This method has been used to detect the following: (1) nicotine and its more persistent metabolite, cotinine, in the blood and urine of cigarette smokers; (2) aldrin and dieldrin, which can be detected at picomole levels in human tissues, as well as in food samples; and (3) adriamycin. These studies provide a means for defining exposure in population groups at high cancer risk (Contractor: Brandeis University, NO1-CP-23243, Biology and Immunology Segment).

In a study of carcinogens and immunosuppression, it was found that tumors induced by UV light exhibited an unusually high degree of antigenicity (Contractor, University of Utah, NO1-CP-45600, Biology and Immunology Segment). The findings on the antigenicity of UV-induced tumors provide a unique opportunity to investigate the mechanism which enables certain highly antigenic tumors to escape immunologic destruction in the primary host.

Studies on BCG and on carcinoembryonic antigens have been transferred to the Tumor Immunology Program, Division of Cancer Biology and Diagnosis, NCI.

Studies on rat alpha-fetoglobulins have shown that increased production of alpha-fetoglobulin is not a necessary consequence of malignant transformation of liver cells. On the other hand, alpha-fetoglobulin appears in the serum of rats within one week after feeding certain hepatocarcinogens. Elevation of the serum concentration of alpha-fetoprotein may be used as an accurate index of the growth of a tumor that produces alpha-fetoprotein. As an alpha-fetoprotein-producing tumor grows, the serum concentration of alpha-fetoprotein rises. Removal of the tumor by surgery results in a rapid fall in serum alpha-fetoprotein concentration with a half-life of 24 hours. The presence of occult metastases may be determined by a resumption of rising alpha-fetoprotein serum concentrations in rats whose primary tumor has been removed. This system provides a model for therapy of minimal residual metastatic tumor (Contractor: University of California [at San Diego], NO1-CP-33403, Biology and Immunology Segment).

Implantation of syngenic tumors in mice depress macrophage chemotaxis towards inflammatory stimuli in vivo and in vitro. Patients with locally recurrent malignant melanoma and depressed monocyte chemotaxis had a 50% incidence of

distant metastasis or tumor related death within four months after initial testing. In contrast, patients with locally recurrent malignant melanoma and normal monocyte chemotaxis had only a 13% incidence of deep metastasis or death within the same time period. Patients with depressed monocyte chemotaxis who undergo surgical removal of their tumors develop enhanced chemotactic responsiveness shortly after tumor removal. These findings suggest that tumors themselves may depress monocyte chemotactic function, thus diminishing the localization of macrophages at tumor sites and reducing the ability of a host to destroy neoplastic cells (Duke University Medical Center, NO1-CP-33313, Biology and Immunology Segment).

Line-1 and line-10 guinea pig hepatoma cells, resistant to killing by antibody and guinea pig complement, were made sensitive to immune killing by incubating them with various metabolic inhibitors and chemotherapeutic drugs. The following inhibitors were found effective in increasing cell susceptibility to killing: actinomycin D, puromycin, mitomycin C, hydroxyurea, adriamycin, azacytidine, methotrexate, 5 FU and 6 MP. The effect of these inhibitors was dependent on concentration, temperature, time and was entirely reversible within a few hours after removal of the inhibitors. The mechanisms whereby these inhibitors increase susceptibility to immune killing are not understood and are currently being investigated. Irrespective of mechanism(s), the beneficial effect of various drugs and metabolic inhibitors in the treatment of cancer might be due, in part, to their ability to increase the susceptibility of tumor cells to killing by antibody and complement (Immunochemistry Section, Biology Branch).

Admixture of cells from a guinea pig hepatoma that grows progressively in the skin (line-10) with cells from a hepatoma that grows transiently in the skin (line-1) caused suppression of line-10 cells provided the test animal was not immunized against line-1 cells. In a similar experiment in which the test animals were first immunized against line-1, there was no suppression of line-10 growth (Cellular Immunity Section, Biology Branch).

Systemic administration of pyran copolymer led to inhibition of growth of murine fibrosarcomas. The degree of inhibition of tumor growth appeared to be related to the degree of antigenicity of the tumors as measured by transplantation protection (Cellular Immunity Section, Biology Branch).

Microscopic axillary lymph node metastases were eliminated after injection of BCG into dermal tumors. Optimal eradication of lymph node metastases required that the dermal tumor be infected for at least seven days. Alternative routes of BCG infection were not effective in eliminating microscopic axillary lymph node metastases. Injection of living BCG into tumors growing in the colon wall of guinea pigs completely inhibited tumor growth. Injection of living BCG into the colon of Rhesus monkeys was found to be a safe procedure. Monkeys treated by intracolonic injection of BCG did not develop intestinal obstruction, intestinal bleeding, or perforation (Cellular Immunity Section, Biology Branch).

Injection of living Listeria monocytogenes into established dermal tumors caused tumor regression. In mice, Listeria infection was more effective

than BCG infection in promoting regression of dermal tumors. Evaluation of the effects of Listeria infection in guinea pigs was limited by toxicity (Cellular Immunity Section, Biology Branch).

Considerable new work has been devoted to studies on the mechanisms of immune cell killing and on chemotaxis (Biology Branch). The development of immunologic methods to identify and detect exposure of individuals or population groups to known carcinogens and the development of immunologic measures to inhibit or modify tumor growth are important targets for the inhibition and/or control of cancer. The existing program directions will be continued and developed. As findings emerge from specific areas of activity, they will be pursued by the mechanisms found to be most appropriate.

F. CHEMISTRY, CARCINOGEN METABOLISM, AND MOLECULAR CARCINOGENESIS

The chemistry, reactivity and metabolic products of several categories of chemical carcinogens have been further elucidated. Analytical methods for carcinogen detection have been developed. The systems that control carcinogen activation in target tissues have been further defined. The macromolecular interactions and mechanisms of action of carcinogens have been further studied with particular attention to DNA repair processes and to membrane studies.

(1) N-Nitroso Compounds

A quotation from Sir Richard Doll, reported by P. N. Magee at the Workshop on Nitrosamines, organized by this Program on February 6-7, 1975, vividly synthetizes the current search for the role of nitrosamines in human cancer: "Nitrosamines are carcinogens in search of a human tumor to cause."

A major program on the carcinogenic activity, formation, metabolism, and analytical methods for nitrosamines and related compounds is now well underway. Particular emphasis is being given to the possible in vivo formation of nitrosamines in animals and man, the metabolic fate of the nitrosamines, their reaction with target cell constituents, and their possible involvement in so-called spontaneous tumor development in humans.

Analytical methods for elucidating the environmental distribution of N-nitroso compounds and for understanding the biochemical and biological consequences of exposure to them were not as well defined as desirable. As a result of this identified research need, a coordinated program has been established in close collaboration with staff in FDA who have a direct concern with analytical resources in this area. This program includes activities concerned with (a) improved methodologies for the analysis of volatile nitrosamines, (b) development of methods for the analysis of non-volatile nitrosamine, (c) development of group-specific analytical methodologies suitable for the analysis of total nitrosamine content, (d) improvement of

instrumentation in this analytical area, and (e) preparation and distribution of labeled nitrosamines for the validation of previously developed analytical methods (Contractors: British Food Manufacturing Industries Research, NO1-CP-44337, University of Mississippi, NO1-CP-43347, Oregon State University, NO1-CP-43348, Simon Fraser University, NO1-CP-43338, and Thermo Electron Corp., NO1-CP-45623, Carcinogen Metabolism and Toxicology Segment). Studies in this area are also being pursued in-house in support of these activities (Carcinogen Metabolism and Toxicology Branch) and through other collaborative projects (University of Nebraska [Eppley Institute for Research on Cancer], NO1-CP-33278, and ERDA-Oak Ridge National Laboratory, YO1-CP-50200, Office of the Associate Director for Carcinogenesis).

Using a newly developed gas chromatography Thermal Energy Analyzer Interface, analytical procedures, sensitive at the part per trillion level have been developed for the gas chromatograph amenable N-nitroso compounds. This sensitivity is sufficient for metabolic studies. Non-volatile N-nitroso compounds can now be determined in nanogram amounts using a newly developed high performance liquid chromatography Thermal Energy Analyzer interface. Although the new technique is still in its infancy, it is the only method currently available which can be used for the analysis of the N-nitroso ureas and similar compounds (Contractor: Thermo Electron Corp. NO1-CP-45623).

Methods for sampling, concentration and detection of trace levels of volatile carcinogens in air are under development. Metal tubes, packed with a porous polymer (tenax-GC), concentrate the components of interest while minimizing interference from water vapor. Use of these tubes permits remote sampling and storage of collected samples prior to analysis. Fluorocarbon permeation tubes are employed for production of standard atmospheres contaminated with low levels of nitrosamines. Gas chromatography, employing the Hall electrolytic conductivity detector in the nitrogen mode is used for detection and quantitative measurements with confirmation of identity by combined gas chromatography-mass spectrometry. At the current stage of development, dimethylnitrosamine and diethylnitrosamine can be measured readily at the one part per billion level in air. This method will be applied to monitoring of laboratory and animal room atmosphere (Contractor: University of Nebraska [Eppley Institute for Research on Cancer], NO1-CP-33278, Office of the Associate Director for Carcinogenesis).

The possible formation of nitrosamines in the environment, and particularly in foodstuffs during preparation and storage or from endogenous materials consumed with the diet is being investigated (Contractor: Massachusetts Institute of Technology, NO1-CP-33315, Carcinogen Metabolism and Toxicology Segment). This effort has shown that the food additive, sodium nitrite, can react with other food constituents under a variety of conditions of temperature and pH to form some nitrosamines.

Kinetics of the nitrosation reaction are receiving detailed study (Contractor: University of Nebraska [Eppley Institute for Research on Cancer], NO1-CP-33278, Office of the Associate Director). A related study is being conducted

with emphasis on the formation of nitrosamines from commonly used tertiary amine drugs (Contractor: ERDA-Oak Ridge National Laboratory, YO1-CP-50200, Office of the Associate Director).

The significance of the presence of nitrite in human saliva is being investigated. Salivary nitrites are probably the greatest contribution to human exposure. Since nitrate is a constituent of many foods, the implication of its reduction to nitrite and further reaction with dietary amines is obvious. Nitrosamine formation has been demonstrated in human saliva using morpholine, diethylamine, and dimethylamine as precursors. Rates of formation are too high to be explained by simple chemical reaction with nitrite, and microbial catalysis is implicated (Contractor: Massachusetts Institute of Technology, NO1-CP-33315, Carcinogen Metabolism and Toxicology Segment).

Investigations are being made of naturally-occurring compounds which may inhibit the formation of nitrosamines from nitrite. While it was confirmed that ascorbic acid in the diet drastically inhibits lung adenoma induction by nitrite given with piperazine, morpholine, or methylurea, it was found, however, that ascorbic acid produced 15-59% increases in adenoma induction by two preformed nitrosamines. Substituted phenols are major components of hardwood smokes, which are applied to many nitrite-cured foods. Reaction of phenols with nitrite to form nitrosophenols and other products could compete with nitrosation of amines to inhibit N-nitroso compound formation. In model reaction mixtures, 2,6-dimethoxyphenol (syringol), the major phenolic component of wood smoke, provided 92% blockage, of nitrosomorpholine production from nitrite and morpholine at pH 3. The extent of blockage was equivalent to that produced by an equivalent concentration of ascorbic acid. Some other phenolic components of wood smoke which blocked nitrosomorpholine formation were phenol (15%), guaiacol (71%), and 4-methylguaiacol (81%) (Contractor: University of Nebraska [Eppley Institute for Research on Cancer], NO1-CP-33278, Office of the Associate Director for Carcinogenesis).

The hypothesis that methyl(hydroxymethyl) nitrosamine is a carcinogenic metabolite of dimethylnitrosoamine (DMN) was tested by synthesizing the acetyl ester and the methoxy derivatives: while both DMN and methoxy-DMN induced renal tumors in rats by single intraperitoneal injection, acetoxy-DMN induced no renal tumors but multiple polyps and carcinomas in the small intestine and colon (Carcinogen Metabolism and Toxicology Branch and Experimental Pathology Branch).

(2) Polynuclear Hydrocarbons

An extensive effort has been devoted in the Chemistry Branch to studies on the metabolism of polynuclear aromatic hydrocarbons (PAH), a class of chemical carcinogens present in organic combustion products known to be carcinogenic in man, such as tobacco smoke, pitch, tars, soot, and petroleum combustion products. Some of these studies involve the mechanism of interaction and the effects of certain co-factors on the metabolism of polynuclear hydrocarbons.

One of the major difficulties in understanding the mechanisms of polynuclear hydrocarbon carcinogenesis has been the difficulty of analysis and quantitation of active and inactive metabolites formed from the precursor molecules. These methods in the past have been extremely laborious and so inaccurate as to make quantitation essentially impossible. In the last year a critical advance in this methodology has occurred which will permit workers in the field of polycyclic hydrocarbon carcinogenesis to take major steps forward. This advance is based on the use of high pressure liquid chromatography which can accurately, clearly, and quantitatively separate at least ten metabolites of benzo[a]pyrene in a short time (60 minutes). This technique is now being applied to the study of tissue, species, and genetic differences in metabolism. High pressure liquid chromatography has been applied to the analyses of benzo[a]pyrene metabolites formed by human liver and lymphocytes. At least four new metabolites have been detected. Methods have also been developed for the isolation of benzo[a]-pyrene epoxides, which may be key carcinogenic intermediates (Molecular Carcinogenesis Section, Chemistry Branch).

With this technique, tissue susceptibility to carcinogens can be correlated with metabolic profiles thus permitting identification of those metabolites which are detoxification products and those which are active forms of the carcinogens (Chemistry Branch). Metabolites and metabolic pathways have been further defined in several other projects, and a major effort has been made to synthesize reference compounds (Chemistry and Molecular Carcinogenesis Segment).

Extensive studies are also being conducted on the enzyme systems which control metabolic detoxification and activation of PAH. More sensitive methods to detect the enzymes involved in easily obtainable human tissue such as blood and skin are now being actively developed and refined so that the test can be reproducibly applied to the screening of human populations. These developments may enable us to determine the carcinogen metabolizing ability of various human populations. A relationship may exist between the incidence of certain chemically-induced cancers in humans and the PAH metabolizing activity of the populations studied.

The large number of compounds when introduced into the organism are metabolized by the microsomal mixed function oxygenase enzyme system. This enzyme system is influenced by a variety of environmental factors such as previous exposure to drugs, pesticides or carcinogens, nutritional and hormonal state, age, sex, and genetic makeup of the organism. A number of substances can modify carcinogenic response to PAH either by inducing the aryl hydrocarbon hydroxylase (AHH) enzyme to high levels or by inhibiting it. It has been found that AHH activity is present in a variety of lymphoid tissues in guinea pigs and that it can be localized in peritoneal and alveolar macrophages, but not in lymph node lymphocytes. In the case of benzo[a]pyrene, the metabolic pathway and the mechanism by which modifiers of this enzyme system change the metabolic profile has been defined (Biology Branch and Chemistry Branch). AHH has been analyzed in human monocytes, in which half life of the enzyme was found to be four to five hours. Induction of the enzyme in different individuals ranged from 7-20 fold. An assay

method has been developed for the analyses of benzo[a]pyrene-4,5-oxide hydratase, an enzyme converting arene oxides to the corresponding dihydrodiols. An assay has been developed for the measurement of glutathione-S-benzo[a]pyrene-4,5-oxide transferase. This is a key enzyme that conjugates reactive epoxides of benzo[a]pyrene and may be instrumental in detoxification processes (Molecular Carcinogenesis Section, Chemistry Branch).

(3) Other Carcinogens

Other efforts on the metabolism of carcinogens are involved with aflatoxins and other mycotoxins which have been identified as contaminants of foodstuffs. The metabolism of aflatoxin in vivo, its effects on cell systems, and its in vitro metabolism in tissues from various species, including man, are being studied. Several mycotoxins found in foodstuffs have an extremely powerful physiologic action. A number of these have carcinogenic activity and others are under investigation (Contractors: Massachusetts Institute of Technology, NO1-CP-43265 and NO1-CP-33238, Carcinogen Metabolism and Toxicology Segment).

Other metabolic studies were developed on aromatic amines (Carcinogen Metabolism and Toxicology Branch), on tryptophan metabolites (Contractor: NCI/FCRC [Litton Bionetics, Inc.], NO1-CO-25423, Office of the Associate Director Carcinogenesis), on DDT and its metabolites in different species (Contractor: University of Nebraska [Eppley Institute for Research on Cancer], NO1-CP-33278, Office of the Associate Director for Carcinogenesis) and on other chemicals related to different research programs.

(4) Molecular Control Mechanisms

Studies on DNA repair synthesis have continued in the Chemistry Branch and in the Experimental Pathology Branch.

Continuing studies into the mechanisms of DNA repair in mammalian cells have shown that the repairability of DNA lesions induced by carcinogens in various cell lines fall into two classes, those which are UV-like and those which are X-ray-like. Normal human cells can excise essentially all UV-induced pyrimidine dimers if the dose of UV is small enough. In studies using fish cells and UV photoreactivation, the formation of pyrimidine dimers in DNA was found to be an initial alteration capable of inducing malignant transformation. This finding is of particular interest as it illustrates in a specific fashion the role of the genome in the development of cancer (Contractor: ERDA-Oak Ridge National Laboratory, YO1-CP-50200, Office of the Associate Director for Carcinogenesis).

It has been found that viral DNA recombination with host genome is increased by carcinogens (Contractor: Weizmann Institute of Science, NO1-CP-33220, Chemistry and Molecular Carcinogenesis Segment).

Investigations are underway for the study of several molecular changes resulting from exposure to chemical carcinogens. The activation of carcinogens results in direct chemical binding to DNA: studies are in progress on the kind of bonds formed (Contractor: Institute for Cancer Research, NO1-CP-33367, Chemistry and Molecular Carcinogenesis Segment). Investigations are in progress on mutations in the cell cycle which can be detected by measurements of the growth phases of normal and chemically transformed cells (Contractor: Salk Institute, NO1-CP-33405, Office of the Associate Director). The first findings indicate that the controls expressed by normal cells in the timing of different phases of the cell cycle are lost upon chemical transformation and cell division proceeds in an unregulated manner.

Projects are now underway for detailed studies on the changes in cell-cell interactions upon transformation (Contractor: Hebrew University, NO1-CP-43307, Chemistry and Molecular Carcinogenesis Segment), on molecular and kinetic changes in membranes of mammalian cells following transformation (Contractor: University of Rochester, NO1-CP-45611, Chemistry and Molecular Carcinogenesis Segment), and on physiological changes and chemical changes in mitochondrial membrane systems and plasma membranes in chemically-transformed cells (Contractor: Johns Hopkins University, NO1-CP-45610, Chemistry and Molecular Carcinogenesis Segment).

A definition of the key activation steps which are obligatory precursors to the advent of the carcinogenic response is highly relevant and brings us closer to having available an exploitable mechanism for the prevention of cancer resulting from environmental, occupational, and dietary carcinogens. Elucidation of such key metabolic steps is also an essential requirement for comparing the carcinogenic response of man to that of animals. Comparative metabolic studies are one of the critical needs for an evaluation of risk in man following exposure to environmental chemicals known to be carcinogenic in animals. Extension of these studies and of their applicability to man is proposed.

* * * * * * *

CONCLUDING REMARKS

The complexity and extent of the advances made during FY 1975 in the Carcinogenesis Program defies summarization. Activities are underway using a large number of research methodologies and biological models at the molecular, cellular, and tissue levels. This document has attempted to identify at least the key areas in which significant advances have been made.

A balance sheet of the accomplishments and of the problems of this Program in this fiscal year can only point out the widening discrepancy between increasing responsibilities--in terms of responsiveness to program needs, of scientific program management, of budget and research projects--on one hand; and the meager and even decreasing support received for personnel allocations to carry it all out, on the other hand.

Inadequate space allocations, increasingly complicated bureaucratic procedures and lack of timely support in many critical areas of program need have severely damaged the efficiency of this Program. The assignment of responsibilities for certain individual activities, which are an integral part of a concerted effort in carcinogenesis, to some offices other than those in the Program, has made the operation of a cohesive effort even more difficult.

The severe problems documented in this respect in the past few years have remained and often have worsened. The recently established Subcommittee on Environmental Carcinogenesis of the National Cancer Advisory Board has noted the incongruity between the wide national recognition of a high priority and urgency for an expanded effort in carcinogenesis and the neglect for adequate qualified support to staff responsible for its implementation within the resources of NCI.

In spite of these persistent and often discouraging difficulties, the Carcinogenesis Program staff has given once more, in this fiscal year, an outstanding, dedicated support to the Program's activities, struggling to maintain high standards of scientific excellence in intramural research and a responsible, scientifically qualified coordination of collaborative research: The accomplishments of each organizational component of the Carcinogenesis Program, and of the individual investigators in the staff, described in the following sections of this report, speak for themselves.

4. FISCAL INFORMATION

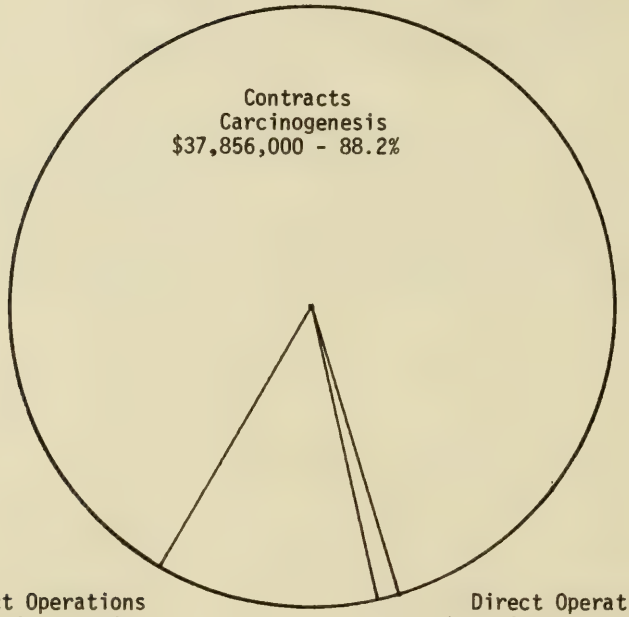
The following charts and tables report the fiscal history and the present distribution of funds in the Carcinogenesis Program, including Direct Operations and Contracts:

Allocations - Direct Operations and Contracts
Distribution of Contract Funds
Fiscal History
Trend in Budget and Positions
Table I - Analysis of Contract Activities by Segment
Table II - Analysis of Contracts by Activity

CARCINOGENESIS PROGRAM

Allocations - Direct Operations and Contracts[1]

Fiscal Year 1975



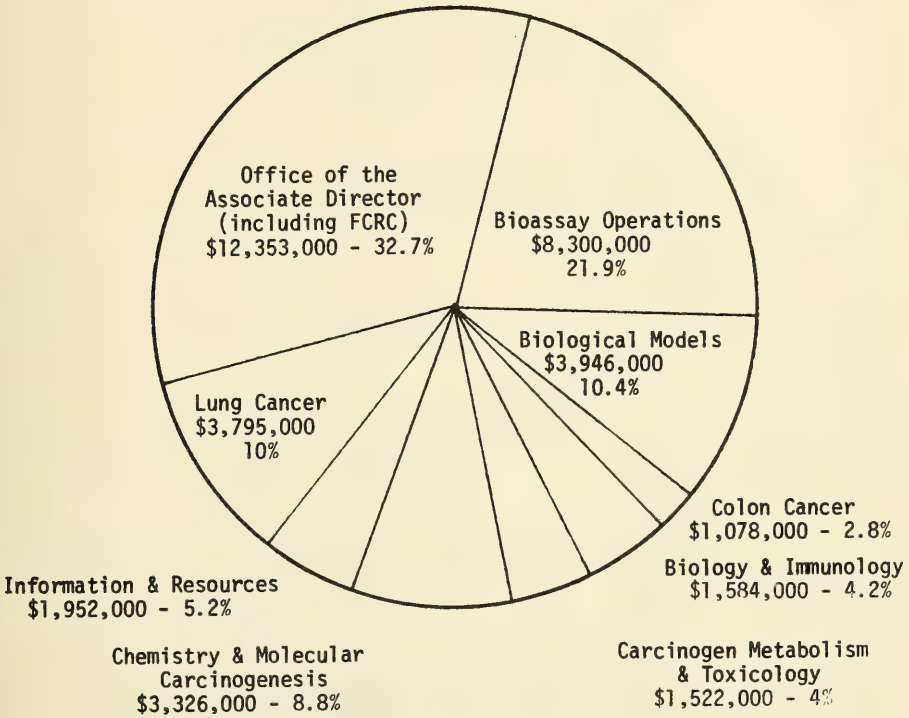
Total Contracts:	$37,856,000
Total Direct Operations:	5,068,000
TOTAL FUNDING:	$42,924,000

[1]Estimation based on information available as of March 31, 1975.

CARCINOGENESIS PROGRAM

Distribution of Contract Funds[1]

Fiscal Year 1975


Office of the Associate Director (including FCRC) $12,353,000 - 32.7%
Bioassay Operations $8,300,000 21.9%
Biological Models $3,946,000 10.4%
Lung Cancer $3,795,000 10%
Colon Cancer $1,078,000 - 2.8%
Biology & Immunology $1,584,000 - 4.2%
Information & Resources $1,952,000 - 5.2%
Chemistry & Molecular Carcinogenesis $3,326,000 - 8.8%
Carcinogen Metabolism & Toxicology $1,522,000 - 4%

Total Contract Funds: $37,856,000

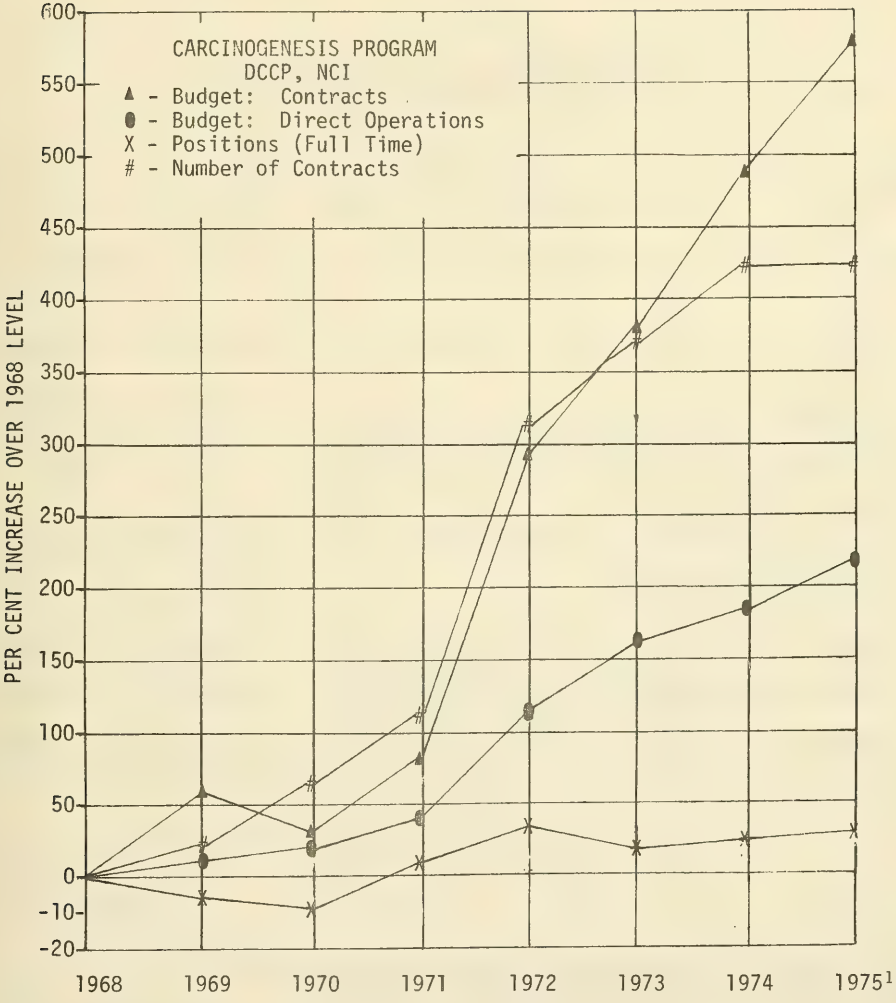
[1]Estimation based on information available as of March 31, 1975.

CARCINOGENESIS PROGRAM - FISCAL HISTORY

DESCRIPTION	JUNE 1968	JUNE 1969	JUNE 1970	JUNE 1971	JUNE 1972	JUNE 1973	JUNE 1974	PROJECTED JUNE 1975
Personnel[1]								
Full-Time Permanent	107	98	96	110	145	138	139	140
Other Full-Time	1	1	8	9	23	10	7	10
Part Time	1	3	2	7	12	19	21	25
Total	109	102	106	126	180	167	173	175
Space	15,885	26,829	26,112	28,337	31,041	38,164	38,075	38,075
Budget								
Direct Operations	$1,636,000	$ 1,802,600	$1,997,000	$ 2,340,000	$ 3,538,000	$ 4,212,000	$ 4,701,000	$ 5,068,000
Contracts	5,602,000	8,466,600	7,080,000	10,598,000	21,985,813	26,964,819	33,056,250	37,856,000
Total	$7,238,000	$10,269,200	$9,077,000	$12,938,000	$25,523,813	$31,176,819	$37,757,250	$42,924,000
Number of Contracts	33	40	51	69	133	154	175	175

[1] Includes all personnel actually assigned to the Carcinogenesis Program.

TREND IN BUDGET AND POSITIONS



[1]Estimation based on information available as of March 31, 1975.

TABLE I

ANALYSIS OF CONTRACT ACTIVITIES BY SEGMENT
IN THE CARCINOGENESIS PROGRAM

SEGMENT	NO. OF CONTRACTS	TOTAL AMOUNT[1]	PERCENT
TOTAL	175[2]	$37,856,000	(100%)
1. OADC	18	12,353,000	32.7
2. Bioassay Operations	7	8,300,000	21.9
3. Biological Models	35	3,946,000	10.4
4. Biology and Immunology	16	1,584,000	4.2
5. Carcinogen Metabolism and Toxicology	10	1,522,000	4.0
6. Chemistry and Molecular Carcinogenesis	32	3,326,000	8.8
7. Colon Cancer	9	1,078,000	2.8
8. Information and Resources	29	1,952,000	5.2
9. Lung Cancer	19	3,795,000	10.0

[1]Estimation based on information available as of March 31, 1975
[2]Several contracts are listed more than one time.

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
1. OFFICE OF THE ASSOCIATE DIRECTOR FOR CARCINOGENESIS		($12,353,000)
AMERICAN HEALTH FOUNDATION NO1-CP-33208	Supplemental Support to OD, DCCP	470,617
ARMY, DEPARTMENT OF (Interagency Agreement with Ft. Detrick) YO1-CP-50004	Support Agreement to FCRC	1,066,000
CHARLES RIVER BREEDING LABS. NO1-CM-53812	Partial Support to DCT	100,000
COLUMBIA UNIVERSITY NO1-CP-23234	Development of a Tissue Culture Transformation System for Aromatic Amine Carcinogens	192,000
ERDA-NCI INTERAGENCY AGREEMENT (Oak Ridge National Lab.) YO1-CP-50200	NCI-ERDA Carcinogenesis Program	[2]
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER NO1-CP-55630	Evaluation of the Significance of Experimental Chemical Carcinogenesis Data to Man	134,900
MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES NO1-CP-23286	Oncogenesis and Other Late Effects of Cancer Therapy	100,000
NCI, FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) NO1-CO-25423	Operation and Maintenance of the NCI's Frederick Cancer Research Center	5,795,730
NEBRASKA, UNIVERSITY OF (Eppley Institute for Research on Cancer) NO1-CP-33278	A Resource for Carcinogenesis Bioassays and Related Research	2,993,753

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
SALK INSTITUTE NO1-CP-33405	Carcinogenesis and Control of Cell Cycle	-0-
TO BE AWARDED	Development of Methods and Protocols for Carcinogen Screening Using Cell Culture Assays	1,500,000
2. BIOASSAY OPERATIONS SEGMENT		($8,300,000)
CINCINNATI, UNIVERSITY OF NO1-CP-33202	Study of the Carcinogenic and Co-Carcinogenic Properties of Industrial Chemicals	78,641
HOWARD UNIVERSITY NO1-CP-33266	Chemical and Biological Investigation of Potential Carcinogens from Plants	150,000
NCI, FREDERICK CANCER RESEARCH CENTER NO1-CO-25423	Project 8 - Bioassay Research	[3]
NEW YORK UNIVERSITY NO1-CP-43221	Alkylating Agents as Carcinogens	-0-
SAN FRANCISCO, UNIVERSITY OF NO1-CP-33229	Study of the Carcinogenicity of Heavy Metals	20,000
TEMPLE UNIVERSITY (Skin and Cancer Hospital) NO1-CP-43271	UV Photocarcinogenesis: Effects of Depletion of Atmospheric Ozone	147,577
TRACOR JITCO, INC. NO1-CP-43350	Bioassay Operations Prime Contract	7,500,000
TO BE AWARDED	Development of Operations Manual for Bioassay	100,000
TO BE AWARDED	Bioassay Resources	303,782

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
3. BIOLOGICAL MODELS SEGMENT		($3,946,000)
ALTON OCHSNER MEDICAL FDN. NO1-CP-43229	Carcinogenesis by Radiation Plus Estrogen	94,000
BIOTECH RESEARCH LABS., INC. NO1-CP-55640	Endpoints for In Vitro Carcinogenesis without Transplantation to Syngeneic Hosts	121,143
BOSTON UNIVERSITY NO1-CP-23297	Study of Chemically Induced Carcinogenesis of the Pancreas	42,085
CASE WESTERN RESERVE UNIVERSITY NO1-CP-23284	Enhanced Induction of Guinea Pig Pancreatic Adenocarcinoma	145,000
CHICAGO, UNIVERSITY OF NO1-CP-43317	Definition of Sensitivity of Carcinogenesis Bioassay Systems	68,159
CHICAGO, UNIVERSITY OF NO1-CP-33376	Route of Carcinogen Administration in Pancreatic Adenocarcinoma Induction	-0-
CHILDREN'S HOSPITAL OF LOS ANGELES NO1-CP-55641	Fibrinolysis as a Parameter of In Vitro Transformation	46,818
CHILDREN'S HOSPITAL RESEARCH FDN. NO1-CP-33307	Study the Etiology of Medulloblastoma and Other Brain Tumors by a Combination of Teratogenic and Transplantation Oncogenic Treatments	97,790
CORBEL LABS. NO1-CP-23299	Provide Animal Holding Facilities and Service	15,701
DARTMOUTH COLLEGE NO1-CP-33378	Enhanced Delivery of Synthetic Nitroso Compounds to the Pancreas in Rats	121,115
ERDA-NCI INTERAGENCY AGREEMENT (Brookhaven National Lab.) YO1-CP-30213	Synergistic Interaction of Hormones and Neutron Radiation on Mammary Gland Carcinogenesis	-0-

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
HAZLETON LABS. NO1-CP-23287	Induction of Adenocarcinoma of the Prostate	-0-
KANSAS, UNIVERSITY OF NO1-CP-23271	Histogenesis of Guinea Pig Pancreatic Adenocarcinoma	38,010
LITTON BIONETICS, INC. NO1-CP-22063	Resource for Preparation and Examination of Experimental Biological Material for Histological and Ultra-structural Study	133,141
MARYLAND, UNIVERSITY OF NO1-CP-23206	Studies of the Histogenesis of Renal Carcinoma	91,957
MASON RESEARCH INSTITUTE NO1-CP-23238	Study of the Uptake and Excretion of Carcinogens in and Their Effect on the Prostate	57,233
MELOY LABS., INC. NO1-CP-55613	Transplacental Carcinogenesis in Erythrocebus patas	146,331
MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES NO1-CP-43232	Identification of Site of Origin of Human Pancreatic Adenocarcinoma	-0-
MICHIGAN, UNIVERSITY OF NO1-CP-45619	Purification of Epidermal Chalone, A Tissue Specific Inhibitor of Cellular Proliferation	112,000
MICHIGAN, UNIVERSITY OF NO1-CP-33331	Organ Culture of the Prostate: A Biological Model	138,542
MICROBIOLOGICAL ASSOCIATES, INC. NO1-CP-02199	Laboratory Service for Support in Carcinogenesis Bioassay and Related Activities	900,000
NEBRASKA, UNIVERSITY OF (at Lincoln) NO1-CP-33289	Chemical Carcinogen-Induced Noduligenesis and Tumorigenesis in Whole Mouse Mammary Gland Organ Culture	17,700

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
ORGANIZATION FOR HEALTH RESEARCH (TNO) NO1-CP-33330	Synergistic Interaction of Hormones and Neutron Radiation on Mammary Gland Carcinogenesis	-0-
PORTLAND STATE UNIVERSITY NO1-CP-43256	Isolation and Maintenance of Normal Human Prostatic Epithelial Cell Lines	50,000
SOUTHWEST FDN. FOR RESEARCH AND EDUCATION NO1-CP-33379	Gonadal Hormone Effects on the Prostate	149,846
STANFORD UNIVERSITY NO1-CP-55649	The Role of Hormonal Induction of Permanent Cellular Alterations During Perinatal Exposure on Carcinogenesis	72,589
TEMPLE UNIVERSITY NO1-CP-33262	Biochemical and Morphological Components of Hepatic Carcinogenesis	48,238
TENNESSEE, UNIVERSITY OF NO1-CP-33369	Maintenance of Organ Explants from Rodent Pancreas	76,094
WEST VIRGINIA UNIVERSITY NO1-CP-43230	Relationships of Pituitary Hormones and Androgens on Prostate Metabolism	111,066
WRIGHT STATE UNIVERSITY NO1-CP-43231	Cell Culture Development of Human and Guinea Pig Pancreas Cells	56,679
TO BE AWARDED	Potential for Carcinogen Metabolic Activation in the Pancreas of Experimental Animals	200,263
TO BE AWARDED	Reflux as a Mechanism for Induction of Adenocarcinoma of the Pancreas	150,000
TO BE AWARDED	Chemical Structure Requirements	170,000
TO BE AWARDED	Glucuronidases and Sulfatases in Pancreas	200,000
TO BE AWARDED	Endocrine Rhythms in Populations	220,000

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT [1]
TO BE AWARDED	Role of Hormonal Induction of Permanent Cellular Alterations during Perinatal Exposure on Carcinogenesis	54,500
4. BIOLOGY AND IMMUNOLOGY SEGMENT		($1,584,000)
BIOLABS., INC. NO1-CP-45615	In Vitro Study of the Nature of Interaction between Chemical and Viral Carcinogens	30,000
BRANDEIS UNIVERSITY NO1-CP-23243	Production and Detection of Antibodies to Chemical Carcinogens and Other Small Molecules	-0-
CALIFORNIA, UNIVERSITY OF (at San Diego) NO1-CP-33403	Significance and Relationship of Fetoglobulins to the Induction of Hepatomas by Chemical Carcinogenesis	241,000
CASE WESTERN RESERVE UNIVERSITY NO1-CP-43354	Specific Immunological Unresponsiveness to Chemical Carcinogenesis and Its Influence on Tumorigenesis	140,000
CHILDREN'S HOSPITAL MEDICAL CENTER NO1-CP-45612	Effects of Carcinogens on In Vitro Synthesis of Complement Components	73,778
DUKE UNIVERSITY MEDICAL CENTER NO1-CP-33313	Response of Peripheral Blood Monocytes from Patients with Neoplastic Disease to Chemotactic Factors	124,000
HAMILTON BIOCHEMICAL RESEARCH LAB. NO1-CP-45609	Supply P3 and Cell Walls from Mycobacterium Bovis Strain BCG	25,000
ILLINOIS, UNIVERSITY OF NO1-CP-23205	Transfer of Tumor Immunity by Cell-Free Extracts of Immune Lymphoid Cells	-0-
MALLORY INSTITUTE OF PATHOLOGY FDN. NO1-CP-33264	Detection of Carcinoembryonic Antigens in Humans	-0-

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
OHIO STATE UNIVERSITY NO1-CP-53329	Chemical Carcinogenesis and Immunity	187,833
OHIO STATE UNIVERSITY RESEARCH FDN. NO1-CP-43276	In Vitro Study of the Nature of Interaction between Chemical and Viral Carcinogens	131,849
PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK, INC. NO1-CP-55679	Chemical Carcinogenesis and Immunity	72,940
SCRIPPS CLINIC AND RESEARCH FDN. NO1-CP-43277	Isolation and Chemical Characterization of Soluble Human Tumor (CEA) Specific Antigens	-0-
TEMPLE UNIVERSITY NO1-CP-33200	Induction of Malignant Melanoma in Guinea Pigs	61,422
TEXAS, UNIVERSITY OF (at San Antonio) NO1-CP-43353	Development of In Vitro Methods for the Detection of Cell-Mediated Immunologic Reactivity to Chemical Carcinogens	85,797
TRUDEAU INSTITUTE, INC. NO1-CP-23221	Tumor Inhibition by Mycobacteria: Standardization of Mycobacteria Preparations	-0-
UTAH, UNIVERSITY OF NO1-CP-45600	Carcinogenesis Bioassay Resource for Determining the Effect of Chronic Immunosuppression on Physical and Chemical Carcinogenesis	275,000
TO BE AWARDED	Studies on an In Vivo/In Vitro System as a Potential Bioassay for Chemical Carcinogens	135,381
5. CARCINOGEN METABOLISM AND TOXICOLOGY SEGMENT		($1,522,000)
ARTHUR D. LITTLE, INC. NO1-CP-33392	Use of Lymphoma Cells In Vitro and in Host-Mediated Bioassays as a Potential Prescreen for Chemical Carcinogens	144,047

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH NO1-CP-44337	Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment	30,000
CALIFORNIA, UNIVERSITY OF (at San Diego) NO1-CP-33232	Pulmonary Tumors in Mice for Carcinogenic and Co-Carcinogenic Bioassay	143,645
COLUMBIA UNIVERSITY NO1-CP-33395	In Vitro and Host-Mediated Microbial Mutagenicity as a Prescreen for Chemical Carcinogens	77,330
ERDA-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) YO1-CP-50200	NCI-ERDA Carcinogenesis Program	350,000
MASSACHUSETTS INSTITUTE OF TECHNOLOGY NO1-CP-43265	Toxicity and Carcinogenicity Associated with Fungal Growth on Foodstuffs	-0-
MASSACHUSETTS INSTITUTE OF TECHNOLOGY NO1-CP-33238	Interactions between Diet and Chemical Carcinogenesis: A Bioassay System	126,755
MASSACHUSETTS INSTITUTE OF TECHNOLOGY NO1-CP-33315	Environmental Occurrence of N-Nitroso Compounds	-0-
MISSISSIPPI, UNIVERSITY OF (School of Pharmacy) NO1-CP-43347	Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment	21,000
OREGON STATE UNIVERSITY NO1-CP-43348	Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment	65,000
SIMON FRASER UNIVERSITY NO1-CP-43338	Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment	15,000

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
STANFORD RESEARCH INSTITUTE NO1-CP-33394	Potential Prescreen for Chemical Carcinogens	159,095
THERMO ELECTRON CORP. NO1-CP-45623	Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment	91,200
TO BE AWARDED	Metabolism of Carcinogenic Compounds	298,928
6. CHEMISTRY AND MOLECULAR CARCINOGENESIS SEGMENT		($3,326,000)
ALBERT EINSTEIN COLLEGE OF MEDICINE NO1-CP-45606	Studies on Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans	243,628
CALIFORNIA, UNIVERSITY OF (at San Francisco) NO1-CP-43239	Selection and Propagation of Somatic Cells Having Specific Physiological Mutations	106,628
CHICAGO, UNIVERSITY OF NO1-CP-33385	Synthesis of Derivatives of Carcinogenic Polycyclic Hydrocarbons	82,000
CONNECTICUT, UNIVERSITY OF (at Storrs) NO1-CP-23268	Isolation and Characterization of Non-Histone Proteins Binding DNA	-0-
ERDA-NCI INTERAGENCY AGREEMENT (California, University of [at Berkeley]) YO1-CP-10058	Molecular Processes Involved in the Carcinogenic Action of Polycyclic Aromatic Hydrocarbons	90,000
ERDA-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) YO1-CP-50200	NCI-ERDA Carcinogenesis Program	90,000
HEALTH RESEARCH INC./ ROSWELL PARK MEMORIAL INSTITUTE NO1-CP-55629	Aryl Hydrocarbon Hydroxylase in Human Lymphocytes, and the Relationship to Chemical Carcinogenesis	62,000

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
HEBREW UNIVERSITY NO1-CP-43307	Mammalian Cell Transport Systems: (3) Influence of Intercellular Factors on Plasma Membrane Transport Systems	80,000
ILLINOIS, UNIVERSITY OF NO1-CP-23303	Temperature Sensitive Mutants in In Vitro Carcinogenesis	165,000
INSTITUTE OF CANCER RESEARCH NO1-CP-33367	Nature of the Polycyclic Hydrocarbon--Nucleic Acid Compound in Hydrocarbon Carcinogenesis	-0-
JOHNS HOPKINS UNIVERSITY NO1-CP-45610	Mammalian Cell Transport Systems: (1) Influence on Mitochondrial Membrane Changes on Plasma Membrane Transport	159,000
JOHNS HOPKINS UNIVERSITY NO1-CP-33276	Regulation of the Heme Moiety of P450 in Relationship to Carcinogen Metabolizing Activity	-0-
MANITOBA, UNIVERSITY OF NO1-CP-43250	Isolation, Purification and Characterization of Human Prolactin	45,326
MICROBIOLOGICAL ASSOCIATES, INC. NO1-CP-55605	Studies on Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans	99,900
MIDWEST RESEARCH INSTITUTE NO1-CP-33387	Organic Synthesis of Cold and Tritiated Polycyclic Hydrocarbon Derivatives	290,000
NEW YORK UNIVERSITY NO1-CP-33279	The Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions	120,000
OHIO STATE UNIVERSITY NO1-CP-43248	Research on Preparation of Polymers Containing Carcinogenic Moieties	31,100
OMNI RESEARCH, INC. NO1-CP-33386	Organic Synthesis of Cold and Tritiated Polycyclic Hydrocarbon Derivatives	30,000

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
ONTARIO CANCER INSTITUTE NO1-CP-43331	The Isolation, Propagation and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions	178,000
POLYSCIENCES, INC. NO1-CP-23245	Optimizing Electrophoretic Separation of Proteins with New Hydrogels	80,000
ROCHESTER, UNIVERSITY OF NO1-CP-45611	Mammalian Cell Transport Systems: (2) Purification of Plasma Membrane Transport Systems	120,000
TEXAS, UNIVERSITY OF (at Austin) NO1-CP-33384	Growth of Soil Bacteria on Polycyclic Hydrocarbons	63,000
TEXAS, UNIVERSITY OF (at Houston) NO1-CP-45604	Studies on Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans	95,400
TEXAS, UNIVERSITY OF (M.D. Anderson Hospital and Tumor Institute) NO1-CP-43270	Non-Histone, DNA Binding Proteins From Normal Rat Liver and Chemically-Induced Rat Hepatomas	93,710
WEIZMANN INSTITUTE OF SCIENCE NO1-CP-33220	Alterations in Translation of Genetic Messages Induced by Viruses and Carcinogens	328,000
WEIZMANN INSTITUTE OF SCIENCE NO1-CP-02217	Study of the Role of Enzyme Induction and Chemical Carcinogenesis	145,000
YALE UNIVERSITY NO1-CP-55673	Selection and Propagation of Haploid and Partial Monosomic Cell Lines	105,551
TO BE AWARDED	Role of Mutation in Carcinogenesis	125,000

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
TO BE AWARDED	Repair Mechanisms in Carcinogenesis: The Use of Fish Cells to Obtain a Quantitative Relation between the Number of Damages to DNA and Neoplastic Transformation	78,300
TO BE AWARDED	Studies on Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans	80,000
TO BE AWARDED	Chemistry Resources	139,457
7. COLON CANCER SEGMENT		($1,078,187)
AMERICAN HEALTH FDN. NO1-CP-43378	Studies in Colon Carcinogenesis	371,000
CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH NO1-CP-33353	Supplement to FSS, DCCP, NCI	27,406
CALIFORNIA, UNIVERSITY OF (at Los Angeles) NO1-CP-43290	Characterization of Mucosal-Associated Microorganisms in the Human Colon and Relationship to Cancer and Precancerous Conditions	75,000
CALIFORNIA, UNIVERSITY OF (at Los Angeles) NO1-CP-33380	Fecal Flora Studies	13,938
GEORGIA, MEDICAL COLLEGE OF NO1-CP-43282	Epidemiologic Study of Colon Cancer among Blacks	55,972
KAISER FDN. RESEARCH INSTITUTE NO1-CP-33215	Epidemiologic Study of Colon Cancer in Blacks	30,320
MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES NO1-CP-43366	Analysis of Regulatory Control of Cell Proliferation in Normal, Premalignant, and Malignant Colonic Tissues (in Familial Polyposis)	171,000

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
MISSOURI, UNIVERSITY OF NO1-CP-33335	Effect of a High Meat Diet on the Bacterial Flora and Chemical Components of Feces	82,246
NCI, FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) NO1-CO-25423	Project 7 - Laboratory Studies of Etiology of Cancer of the Digestive System	[3]
OXFORD, UNIVERSITY OF NO1-CP-23215	Study of the Relationship in Individuals between Colon Cancer and Epidemiologically Linked Diseases	15,000
VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY NO1-CP-33334	Comparative Fecal Flora Studies	236,305
8. INFORMATION AND RESOURCES SEGMENT		($1,952,000)
ASH STEVENS, INC. NO1-CP-23293	Synthesis of Purine and Pyrimidine Nucleotides	-0-
CHARLES RIVER BREEDING LABS. NO1-CP-55651	Establishment of a Gnotobiote Originated Rodent Production Colony	133,177
ERDA-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) Y01-CP-20203	Environmental Mutagen Information Center	80,000
EXPERIMENTAL PATHOLOGY LABS., INC. NO1-CP-43300	Animal Pathology Support	91,000
FRANKLIN INSTITUTE RESEARCH LABS. NO1-CP-55634	Information Resource	201,901
FRANKLIN INSTITUTE RESEARCH LABS. NO1-CP-43293	Carcinogenesis Abstracts, Volume 12-14	15,000

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
IIT RESEARCH INSTITUTE NO1-CP-33296	Production and Characterization of Particulate Materials for Respiratory Studies	-0-
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER NO1-CP-45608	Program for the Evaluation of Carcinogenic Risk of Chemicals to Man	120,000
JOHNS HOPKINS UNIVERSITY NO1-CP-43288	Animal Pathology Support	130,000
MEDIZINISCHE HOCHSCHULE--HANNOVER NO1-CP-12148	Development of a Lung Tumor Model with Large Wild Hamsters (Cricetus cricetus L.)	40,000
MIDWEST RESEARCH INSTITUTE NO1-CP-23270	Analytical Chemistry Resources	-0-
MOUNT SINAI SCHOOL OF MEDICINE NO1-CP-43255	Identification of Human Populations at Risk Due to Exposure to N-Nitroso Compounds	-0-
NATIONAL ACADEMY OF SCIENCES INSTITUTE OF LABORATORY ANIMAL RESOURCES NO1-CP-45617	Partial Support for the Institute of Laboratory Animal Resources	20,000
NCI, FREDERICK CANCER RESEARCH CENTER (Litton Bionetics Inc.) NO1-CO-25423	Project 9 - Preparation and Characterization of Carcinogens	[3]
	Project 12 - Production of Inbred and Hybrid Laboratory Animal Strains	[3]
	Project 15 - Procedures for Safe Handling of Chemical Carcinogens	[3]
NEW HAMPSHIRE, UNIVERSITY OF NO1-CP-55675	Preparation of Various N-Nitroso Compounds	43,374
RESEARCH TRIANGLE INSTITUTE NO1-CP-55671	Preparation of Various N-Nitroso Compounds	90,000

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
STANFORD RESEARCH INSTITUTE NO1-CP-55672	Preparation of Various N-Nitroso Compounds	120,000
STANFORD RESEARCH INSTITUTE NO1-CP-33285	A Research Program to Acquire and Analyze Information on Chemicals that Have Impact on Man and His Environment	84,000
TRACOR JITCO, INC. NO1-CP-33402	Production of 1972-1973 Volume of "Survey of Compounds Which Have Been Tested for Carcinogenic Activity" and Production of Machine Readable and Searchable Indexes of the PHS-149	-0-
WOLF RESEARCH AND DEVELOPMENT CORP. NO1-CP-43257	Carcinogenesis Bioassay Data System (CBDS) Operational Support	203,890
TO BE AWARDED	Chemistry Repository	200,000
TO BE AWARDED	Additional Resources	229,658
TO BE AWARDED	Management Information System	150,000
9. LUNG CANCER SEGMENT		($3,795,000)
ERDA-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) Y01-CP-50200	NCI-ERDA Carcinogenesis Program	776,500
HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH NO1-CP-33273	Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens	140,000
IIT RESEARCH INSTITUTE NO1-CP-43289	Supply of Hamsters Treated with Respiratory Carcinogens for NCI Carcinogenesis Program	151,000
IIT RESEARCH INSTITUTE NO1-CP-23292	Susceptibility States and Modulating Factors in Respiratory Carcinogenesis	399,000

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
LITTON BIONETICS, INC. NO1-CP-43274	Studies of Carcinogenesis in Human Bronchial Tissues	111,205
MARYLAND, UNIVERSITY OF NO1-CP-43237	Studies on Normal, Premalignant and Malignant Tracheobronchial Epithelium of Humans	113,259
MASSACHUSETTS INSTITUTE OF TECHNOLOGY NO1-CP-43253	Role of Vitamin A in the Control of Differentiation and Carcinogenesis in the Respiratory Tract	101,259
MINNESOTA, UNIVERSITY OF NO1-CP-33364	Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract	123,195
NEW YORK UNIVERSITY NO1-CP-33260	Studies in Pulmonary Carcinogenesis	459,555
ST. MARY'S HOSPITAL NO1-CP-23233	Morphogenesis of Lung Cancer	38,642
SOUTHERN RESEARCH INSTITUTE NO1-CP-22064	Organ Culture and Tissue Culture Bioassay of Vitamin A Analogs	254,400
STANFORD UNIVERSITY SCHOOL OF MEDICINE NO1-CP-43295	Studies on Oat-Cell Carcinoma of the Lung	87,862
STATE UNIVERSITY OF NEW YORK (at Stony Brook) NO1-CP-33361	Studies on Carcinogenesis in Organ Culture of Trachea and Bronchi	129,133
STOCKHOLM, UNIVERSITY OF NO1-CP-33363	Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract	179,848
TEXAS, UNIVERSITY OF (Southwestern Medical School at Dallas) NO1-CP-33362	Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract	35,069
VERMONT, UNIVERSITY OF NO1-CP-33360	Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi	114,215

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
VETERANS ADMINISTRATION HOSPITAL (Tampa, Florida) Y01-CP-55625	Autoradiographic Study of the Cellular Response of the Respiratory Tract in Chemical Carcinogenesis	127,485
TO BE AWARDED	Lung Cancer Segment Resources	453,373
GRAND TOTAL		$37,856,000

[1]Total funds estimated based on information available as of March 31, 1975.
[2]Funding is listed under segments supporting various projects at ERDA/ORNL.
[3]Total amount reported under the Office of the Associate Director.

5. OFFICE AND BRANCH REPORTS

SUMMARY REPORT

OFFICE OF THE ASSOCIATE
DIRECTOR FOR CARCINOGENESIS

July 1, 1974 through June 30, 1975

"(1) Plans, implements, and administers coordinated program of basic and applied research on carcinogenesis by chemical and physical factors and on its prevention and (2) conducts programs in the areas of [a] coordination on environmental carcinogenesis and [b] experimental models of cancer of endocrine target organs."

The direct operation of the Office of the Associate Director for Carcinogenesis includes the immediate Office of the Associate Director for Carcinogenesis (U. Saffiotti, Associate Director, and J. A. Cooper II, Deputy Associate Director, C. Warner); the Coordinator for Collaborative Research (A. H. Heim); and the Scientific Coordinator for Carcinogenesis at the NCI's Frederick Cancer Research Center (FCRC) (R. R. Bates). Until August 1974 this Office also included the Tumor Pathology Section which was then transferred to the Experimental Pathology Branch. Until September 1974, this Office also included the activities of a Scientific Coordinator for Environmental Carcinogenesis (H. F. Kraybill), which were established to develop the necessary liaison with other Federal agencies involved in carcinogenesis problems, to provide information on environmental occurrence and exposure to carcinogens and to assist the Associate Director for Carcinogenesis with documentation and response capabilities in relation to increasing demands received by the Carcinogenesis Program from outside sources (e.g. other Federal agencies, Congress, industry, labor, public interest groups, scientific community, and lay public). In September 1974, the Office of the Scientific Coordinator for Environmental Carcinogenesis was transferred administratively from the Carcinogenesis Program to the Office of the Division Director, DCCP. No replacement was made available for the Carcinogenesis staff: the Division is expected to provide the necessary liaison with other Federal agencies.

The planning and coordination of the whole Carcinogenesis Program has continued to take the major effort of the Office. This Office has continued to serve as a focal point for the development of documentation on the assessment of environmental carcinogenesis hazards and on the principles for evaluation of environmental carcinogens, in collaboration with the Field Studies and Statistics Program, with the Director, DCCP, NCI, and with the Director, NCI. However, a more extensive function in this area has been undertaken in this fiscal year by the Office of the Director, DCCP, with the assumption of an Assistant Director (W. G. Flamm) and the transfer of Dr. Kraybill's office, mentioned above.

This Office has participated in a number of review and policy meetings with other Government departments, including the following: the Office of the Assistant Secretary for Health, DHEW; the DHEW Committee to Coordinate Toxicology and Related Programs; the Office of the Commissioner and bureaus of the Food and Drug Administration (FDA); the National Center for Toxicological Research, FDA; the Office of the Administrator and several other offices of Environmental Protection Agency (EPA); the Office of the Director and other offices and divisions of the National Institute of Occupational Safety and Health (NIOSH), the Occupational Safety and Health Administration of the Department of Labor, and other agencies.

Scientific evaluation was provided and testimony was prepared by the Associate Director for the hearings on the pesticides, aldrin and dieldrin, at the request of EPA and for hearings before the Subcommittee on the Environment, Committee on Commerce of the United States Senate.

The Office participated in the activities of the Working Group on the Evaluation of Carcinogenic Risk of Chemicals to Man, International Agency for Research on Cancer, World Health Organization, Lyon, France. This Office also contributed to the organization of several workshops, conferences, and meetings as reported in the Carcinogenesis Program Summary Report.

These and other related activities in the area of evaluation of environmental hazards have required a large share of the resources of this Office, including communication with congressional committees, other Government agencies, the press, and the information offices of NCI, NIH, and DHEW. Major emphasis has been given in all these activities to the unique role of NCI in providing documentation of carcinogenesis bioassay studies and scientific criteria for evaluation of carcinogenic hazards for man.

The major effort of this Office has been devoted to the scientific planning, development, coordination, and implementation of resources and activities assigned to the Carcinogenesis Program.

Office of the Scientific Coordinator for Carcinogenesis at the NCI's Frederick Cancer Research Center - This Office provides scientific coordination for carcinogenesis programs at FCRC which include the following: (a) coordinating the activity of the projects supported by the NCI's Carcinogenesis Program at FCRC, which include: laboratory studies of bowel cancer etiology, large-scale bioassay, preparation and characterization of carcinogens, in vitro bioassay methodology for potential carcinogens, production of inbred and hybrid laboratory animal strains, procedures for safe handling of chemical carcinogens, and chemical carcinogenesis research; (b) collaborating with the Scientific Coordinator for NCI at FCRC, the Associate Director for Carcinogenesis, and Carcinogenesis staff in establishing priorities and levels of funding of projects at FCRC; (c) assuring the establishment and maintenance of standards of excellence in research in the FCRC laboratories through establishment of appropriate review, consultation, and collaborative arrangements and assuring cooperation

among members of various projects in accomplishing their goals; and (d) planning for new projects and developments in carcinogenesis studies.

Research conducted at FCRC under contract support from the Carcinogenesis Program is reported in the section on Collaborative Program Reports.

The small NCI staff working with the Scientific Coordinator for Carcinogenesis at FCRC has continued a laboratory program of intramural research. This is devoted to study of the reaction of carcinogens with nucleophiles. This has a dual purpose: The development of screening tests for potential carcinogens based on their reaction with nucleophiles, and elucidation of the effects of carcinogen interaction of the function of cellular nucleophiles such as DNA. At the present time, the reaction of carcinogens with amino acids is the main approach being investigated for the development of a screening test. The effect of reaction with carcinogens on DNA function is being investigated in a cell free system including DNA from a bacteriophage containing the beta-galactosidase gene and co-factors derived from E. coli deficient in this gene. When phage DNA was reacted with the carcinogen methylsulfate at a concentration of 25 mM and then added to the E. coli protein-synthesizing system which had not been exposed to the chemical, there are a 98% inhibition of activity of newly synthesized beta-galactosidase with only 50% inhibition of RNA synthesis and 30% inhibition of protein synthesis. The mechanism by which enzyme activity is inhibited is under investigation.

Project No. Z01-CP-04696-05-CG
1. Office of the Associate Director for Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Evaluation of Carcinogenic Hazards

Previous Serial Number: Same

Principal Investigator: Umberto Saffiotti, M.D.

Other Investigators: Collaboration from staff of the Carcinogenesis Program, DCCP, NCI

Cooperating Units: None

Man Years:

Total	0.6
Professional	0.3
Other	0.3

Project Description

Objectives:

To examine current knowledge in the field of carcinogenesis and related fields and to identify criteria for the evaluation of carcinogenic hazards by chemical and physical agents and for the prevention of their carcinogenic effects in man. To examine laboratory findings on the biological action of chemicals and to review data on exposures for man in order to formulate a scientific evaluation of their carcinogenic risk.

Methods Employed:

Scientific coordination of intramural and collaborative programs in the Carcinogenesis Program, DCCP, NCI. Consultation with other Government and international agencies and with members of the scientific community. Convening expert advisory groups. Convening of workshops and conferences. Review of scientific information. Individual analysis of data collected in this field and particularly results obtained in carcinogenesis experiments, their pathology and experimental design. Analysis of occupational and environmental exposure data. Analysis of regulatory and legislative provisions.

Major Findings:

Methodology for evaluation of carcinogenic effects was further developed. In particular, contributions were provided to the following activities: (1) the establishment of guidelines for carcinogen bioassay in small rodents (now ready for publication; a workshop was held on this subject) and (2) the development of a coordinated program on in vitro carcinogenesis tests: a workshop on state of the art was held, a laboratory workshop is being planned, and a report was prepared for an international workshop on rapid screening test at the invitation of the International Agency for Research on Cancer.

The program of the Third Annual Carcinogenesis Collaborative Conference was organized as a series of symposia with emphasis on environmental cancer and on bioassay methods, both in vivo and in vitro.

A study was made of the carcinogenesis data and of the pathology submitted by the Environmental Protection Agency on the pesticides, aldrin and dieldrin, mirex, chlordane and heptochlor. An analysis was conducted of the carcinogenesis studies on the detergent additive nitrilotriacetic acid. The state of knowledge on the cancer hazards from benzidine was reviewed and a conference session was organized to define this problem. Several other documentations were studies and reviewed for other Federal agencies (Food and Drug Administration, Environmental Protection Agency, National Institute of Occupational Safety and Health, and the Department of Labor's Occupational Safety and Health Administration).

Numerous contributions have been made towards the establishment of national policies in the field of carcinogenesis hazards, through discussions and documentation provided to congressional committees, and to the Office of Technology Assessment, to DHEW and other Government and international agencies and offices, and to scientific societies.

Participation to the DHEW Committee to Coordinate Toxicology and Related Programs has continued; particular work was devoted to the Subcommittee on Estimation of Risks of Irreversible, Delayed Toxicity.

Significance to Biomedical Research and the Program of the Institute:

The national policies on environmental health and cancer prevention should be based on sound scientific grounds. The Carcinogenesis Program at NCI continued to provide a central source of information and competence in this field. Several Government policy statements issued in this fiscal year by various agencies (e.g. the National Institute of Occupational Safety and Health, the Occupational Safety and Health Administration, and the Environmental Protection Agency) were based on positions and recommendations originated by this Program.

Proposed Course of Project:

Continuation and expansion of these functions.

Honors and Awards

Chairman, Carcinogenesis Contract Program Management Group, DCCP, NCI

Chairman, Carcinogenesis Intramural Program Group, DCCP, NCI

Member, Cause and Prevention Executive Staff, DCCP, NCI

Member, NCI Advisory Committee for the Frederick Cancer Research Center, NCI

Member, Advisory Committee to Scholars-in-Residence Program, Fogarty International Center, NIH

Alternate Member, DHEW Committee to Coordinate Toxicology and Related Programs, Office of the Assistant Secretary for Health, DHEW; and member of its Subcommittees on Estimation of Risk of Irreversible, Delayed Toxicity and on NTA

Observer at the Policy Board and the Scientific Advisory Board meetings of the National Center for Toxicological Research, Food and Drug Administration

Member, Ad Hoc Advisory Group on the Predictive Testing of Chemicals, Office of Technology Assessment, United States Congress, September 1974

Councillor, Society for Occupational and Environmental Health

Member, Editorial Board, Journal of Toxicology and Environmental Health

Invited Lecturer on "Cell Differentiation and Carcinogenesis: Model Studies on Respiratory Epithelia" at the Given Institute of Pathobiology, University of Colorado's Summer Seminar Program on Advances in Cancer Biology (Differentiation and Development), Aspen, Colorado, August 5-9, 1974

Participant and Speaker on "Documentation and Interpretation in Evaluating Carcinogenic Risk", Workshop on Statistical Problems in Developing Procedures for Estimating Risks to Man from the Results of Animal Experiments, sponsored by the National Institute of Environmental Health Sciences, North Carolina, September 30-October 2, 1974

Session Chairman, Seminar on Late Effects of Cancer Therapy, sponsored by the Memorial Sloan-Kettering Cancer Center and the National Cancer Institute, Milan, Italy, October 19, 1974

Invited Speaker on "Experimental Carcinogenesis Testing for Occupational Exposures" in Symposium No. 8 and participant in the XIth International Cancer Congress, Florence, Italy, October 20-26, 1974

Chairman, Session on Vinyl Chloride Toxicology, 2nd International Symposium on Chromosome Aberrations by Industrial Chemicals and Vinyl Chloride Toxicology, sponsored by Medichem, subcommittee of the Permanent Commission

and International Association on Occupational Health, Milan, Italy, October 28-29, 1974

Participant and Speaker, Workshop on Vitamin A and Cancer Prevention, sponsored by the Carcinogenesis Program, DCCP, NCI, November 21-22, 1974

Invited Participant, Workshop on Persons at High Risk of Cancer: An Approach to Cancer Etiology and Control, sponsored by the Field Studies and Statistics Program, DCCP, NCI, and the American Cancer Society, Key Biscayne, Florida, December 10-12, 1974

Session Chairman, Ad Hoc Working Group on Experimental Design for Evaluation of Carcinogenicity of Pesticides, sponsored by the Carcinogenesis Program, DCCP, NCI, January 29-30, 1975

Conference Chairman, Session Coordinator, Panel Chairman, and Speaker at the Third Annual Carcinogenesis Collaborative Conference, Orlando, Florida, February 2-5, 1975

Conference Co-Chairman, Session Chairman, and Speaker, Conference on Occupational Carcinogenesis, organized by the New York Academy of Sciences, and sponsored by Group Health, Inc., the National Cancer Institute, the National Institute for Occupational Safety and Health, and the Society for Occupational and Environmental Health, New York, New York, March 24-27, 1975

Invited Speaker, Ad Hoc Session on Social and Ethical Issues in Cancer Prevention and Therapy, 66th Annual Meeting of the American Association for Cancer Research, San Diego, California, May 8-11, 1975

Invited Speaker, Conference on Nutrition in the Causation of Cancer, sponsored by the National Cancer Institute and the American Cancer Society, Key Biscayne, Florida, May 18-22, 1975

Invited Lecturer, Course of Current Advances in Cancer Epidemiology, sponsored by the International Agency for Research on Cancer, Brussels, Belgium, June 5, 1975

Invited Speaker, Workshop on Rapid Screening Tests to Predict Late Toxic Effects of Environmental Chemicals, sponsored by the International Agency for Research on Cancer and the Commission of European Communities, Brussels, Belgium, June 9-12, 1975

Publications

Saffiotti, U.: Metabolic host factors in carcinogenesis. In Doll, R. and Vodopija, I. (Eds.): Host Environmental Interactions in the Etiology of Cancer in Man. Lyon, France, International Agency for Research on Cancer, IARC Scientific Publications No. 7 and FIC Proceedings No. 18, 1973, pp. 243-252.

Clamon, G., Sporn, M., Smith, J., and Saffiotti, U.: α- and β-retinyl acetate reverse metaplasias of vitamin A deficiency in hamster trachea in organ culture. Nature 250: 64-66, 1974.

Saffiotti, U.: Biological aspects of chemical carcinogenesis: Summary and predictions. In Ts'o, P.O.P. and DiPaolo, J. A. (Eds.): Chemical Carcinogenesis (Part B). New York, Marcel Dekker, Inc., 1974, pp. 729-746.

Montesano, R., Saffiotti, U., Ferrero, A., and Kaufman, D. G.: Syngeristic effects of benzo[a]pyrene and diethylnitrosamine on respiratory carcinogenesis in hamsters. J. Natl. Cancer Inst. 53: 1395-1397, 1974.

Harris, C. C., Kaufman, D. G., Jackson, F. J., Smith, J. M., Dedick, P., and Saffiotti, U.: Atypical cilia in the tracheobronchial epithelium of the hamster during respiratory carcinogenesis. J. Pathol. 114: 17-19, 1974.

Saffiotti, U.: Keynote Address: Progress in respiratory carcinogenesis. In Karbe, E. and Park, J. F. (Eds.): Experimental Lung Cancer. Carcinogenesis and Bioassays. Heidelberg, West Germany, Springer-Verlag, 1974, pp. 1-5.

Sporn, M. B., Clamon, G. H., Smith, J. M., Dunlop, N. M., Newton, D. L., and Saffiotti, U.: The reversal of keratinized squamous metaplastic lesions of vitamin A deficiency in tracheobronchial epithelium by vitamin A and vitamin A analogs in organ culture: A model system for anticarcinogenesis studies. In Karbe, E. and Park, J. F. (Eds.): Experimental Lung Cancer. Carcinogenesis and Bioassays. Heidelberg, West Germany, Springer-Verlag, 1974, pp. 575-582.

Sporn, M. B., Clamon, G. H., Dunlop, N. M., Newton, D. L., Smith, J. M., and Saffiotti, U.: Activity of vitamin A analogues in cell cultures on mouse epidermis and organ cultures of hamster trachea. Nature 253: 47-50, 1975.

Saffiotti, U. and Kaufman, D. G.: Carcinogenesis of laryngeal carcinoma. Laryngoscope 85: 454-467, 1975.

Saffiotti, U.: Experimental carcinogenesis testing for occupational exposures. In Proceedings of the XIth International Cancer Congress (In press).

Saffiotti, U.: Experimental approaches to the identification of environmental carcinogens. In Epstein, S. (Ed.): Environmental Determinants of Human Cancer. (In press).

Saffiotti, U.: Prevention of occupational cancer--toward an integrated program of governmental action: Role of the National Cancer Institute. Ann. N. Y. Acad. Sci. (In press).

Saffiotti, U.: Methodologies for risk assessment in occupational carcinogenesis. Ann. N. Y. Acad. Sci. (In press).

Project No. Z01-CP-04509-02-CG
1. Office of the Associate Director for Carcinogenesis, DCCP
2. Office of the Scientific Coordinator for Carcinogenesis
3. Frederick, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Significance of Chemical-Macromolecular Interactions to Subsequent Cellular Function

Previous Serial Number: Same

Principal Investigator: Beatrice P. Chen, Ph.D.

Other Investigators: H. F. Kung, Ph.D., Richard R. Bates, M.D.

Cooperating Unit: Roche Institute of Molecular Biology
Nutley, New Jersey

Man Years:

Total	:	1.0
Professional	:	1.0
Other	:	0

Project Description

Objectives:

To explore normal and/or altered cellular functions which occur following carcinogen interactions with various cellular components, especially the significance of DNA interaction with carcinogens in the subsequent gene expression in active enzyme synthesis. To utilize the gene directed cell-free enzyme synthesis system to elucidate the mechanisms whereby these interactions affect cellular function; to examine the modifications in cellular function induced by carcinogens which lead to tumorigenesis; to develop means of controlling these altered cellular processes to either prevent or reverse carcinogenesis; and to develop systems which might rapidly and accurately predict carcinogenic potentials of chemicals.

Methods Employed:

A well-defined system where transcription and translation can be controlled is used to examine the effect of carcinogen interaction with DNA and subsequent DNA expression as measured by the synthesis of specific enzyme activity. The *in vitro* synthesis of the enzyme, β-galactosidase, from a bacteriophage DNA (λh80dlacp^S) that contains lac operon is used. This *in vitro* synthesis

system has been modified from the original Zubay's assay by using a total incubation volume of 70 µl and the addition of some other cofactors. Some of the studies further separate the 30,000 xg supernatant (S-30) into 200,000 xg supernatant, ribosomal wash fraction and ribosomes. The DNA following the carcinogen treatment was used to code for β-galactosidase synthesis.

The carcinogen dimethylsulfate (DMS) was used to methylate the DNA. The amount of methylation on DNA was calculated from the radioactivity of ^{3}H-DMS associated with DNA after DNA dialysis and/or CsCl gradient centrifugation.

The effect of DNA methylation on total protein synthesis was studied by using radioactive amino acid incorporation into TCA precipitable material in this system. The total RNA synthesis was done similarly by using labeled ribonucleotide incorporation as a criteria.

Major Findings:

1. The DMS treated DNA was found to be methylated and the amount of methylation was linear with the concentration of DMS up to about 10mM. 0.6% of the nucleotides were methylated at this concentration.

2. The inhibition of the newly synthesized β-galactosidase activity was linear at least to 4mM DMS (50-70% inhibition).

3. The total protein and RNA synthesis was found to be inhibited much less than the β-galactosidase activity. At 25mM DMS concentration while only 2% of β-galactosidase activity was left, 70% of total protein and 50% of total RNA synthetic activity were still present.

Significance to Biomedical Research and the Program of the Institute:

In the search for the mechanism of action of carcinogens, there is little information concerning the exact functional defect caused by those agents. Previously, there have been no studies in a cell-free system to assay the significance of mutagen on carcinogen interaction with DNA, RNA, protein or co-factors independently on the function of a gene as expressed by synthesis of active enzyme. With the system we employed, one can easily replace any post-DNA component along the DNA-RNA-Protein synthesis pathway in this bacterial system. We may determine whether function is specifically impaired as a consequence of chemical binding to any one of those components.

Proposed Course of Project:

1. The methylated DNA will be checked to see if strand breakage had occurred. Other physical means will be tried to see what kind of alterations on DNA had taken place.

2. The impaired β-Gz from carcinogen DMS treated DNA will be studied to ascertain the nature of the inhibition.

3. Other components within the transcription and translation pathway will be tested, such as tRNA, mRNA, on their effects after methylation by DMS.

4. Various carcinogens, potent or weak, will be examined for correlation with their *in vitro* effects in this system.

Publication

Kung, H. F., Brot, N., Spears, C., Chen, B., and Weissbach, H.: Studies on the *in vitro* transcription and translation of the lac operon. Arch. Biochem. Biophys. 160: 168-174, 1974.

Project No. Z01-CP-04599-01-CG
1. Office of the Associate Director for Carcinogenesis, DCCP
2. Office of the Scientific Coordinator for Carcinogenesis
3. Frederick, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Development of Chemical-Reactivity Approaches to Carcinogen Identification

Previous Serial Number: None

Principal Investigator: Richard R. Bates, M.D.

Other Investigators: Jane Idoine

Cooperating Units: Project 16, Frederick Cancer Research Center

Man Years:

Total	:	1.0
Professional	:	1.0
Other	:	0

Project Description

Objectives:

It is now generally recognized that the active forms of chemical carcinogens are electrophiles. Little use has been made of this observation for the purposes of detecting environmental carcinogens. The purpose of this project is to develop practical approaches to carcinogen screening or identification based on the ability of activated chemical carcinogens to react with nucleophiles.

Methods Employed:

The initial approach being used in this new project is to polymerize nucleophilic amino acids into homo or dipolymers attached to a supporting matrix, Sepharose. This material will then be exposed repeatedly to carcinogens or potential carcinogens with or without metabolic activating systems. The insolublized form of polymer attached to the matrix is designed to permit repeated exposure to the carcinogen in order to permit the highest possible levels of reaction to occur. The polymer will then be washed, enzymatically hydrolyzed, and modified amino acids look for.

Major Findings:

At the time of writing, this project is in the early stages. Techniques have been established for polymerizing the amino acids, attaching them to Sepharose, hydrolyzing them with carboxypeptidase and separating products by thin layer chromatography.

Significance to Biomedical Research and the Program of the Institute:

Large sums of money are being spent on whole animal bioassays of environmental chemicals, a high proportion of which turn out to be noncarcinogenic. At the same time resources are not adequate to test many chemicals which should be tested. A cheap, rapid prescreen for carcinogens would greatly enhance the ability to use funds with maximum effectiveness for detecting carcinogens.

Proposed Course of Project:

The next stages of the project will include testing for the reactivity and subsequent ability to detect the products of reaction with direct acting carcinogens. This will be followed by experiments to develop metabolic activation systems to incorporate with the prescreen. Other approaches to detecting reactions between electrophiles and nucleophiles will also be developed.

Honors and Awards

Member, Program Committee, Third International Symposium on Detection and Prevention of Cancer

Invited Participant, Conference on Monitoring the Problem of Mutagenesis and Carcinogenesis in Man, Third Joint Environmental Conference, United States-Japan Cooperative Medical Science Program, August 1974

Member, Meritorious Award Committee, the American Society for Experimental Pathology

Invited Speaker, Conference on Occupational Carcinogenesis, the New York Academy of Sciences, March 1975

Member, Carcinogenesis Contract Program Management Group, Member, Biological Models Segment, Member, Colon Cancer Segment

Publication

Bates, R. R.: Chemical carcinogenesis and the pancreas. J. Surg. Oncol. (In Press).

SUMMARY REPORT

BIOLOGY BRANCH

July 1, 1974 through June 30, 1975

"Plans, develops, and conducts a research program aimed at (1) elucidation of the role of cellular and humoral immune responses in the process of chemical carcinogenesis, its inhibition, and the prevention of cancer growth and spread; (2) biochemical characterization and elucidation of the structure of antigens derived from normal and malignant cells; (3) the acquisition of detailed knowledge of the molecular and cellular events required for cell damage by antibodies and complement, by lymphocytes and macrophages, and by a combination of these humoral and cellular factors; and (4) the development and definition of in vitro cellular transformation systems for the rapid detection of the carcinogenic action of chemicals."

The research program of the Biology Branch is focused on the search for methods to interfere with three stages of the carcinogenic process--(1) transformation of normal to neoplastic cells, (2) multiplication of neoplastic cells to produce a tumor mass, and (3) initiation of metastases. Among the research projects in the Biology Branch are the following: (1) development of short-term and reliable methods, based on in vitro cell transformation, to identify carcinogenic chemicals, (2) development of immunologic methods to identify individuals or population groups at risk to carcinogenesis and (3) development of immunologic methods to kill transformed or neoplastic cells.

Fundamental studies in the four sections of the Biology Branch, Cytogenetics and Cytology, Cellular Immunity, Immunochemistry and Tumor Antigen, have provided the impetus for the initiation of projects designed to reduce human cancer mortality. Basic studies in the Cytogenetics and Cytology Section on the chemically induced transformation of cells in culture to malignant cells have been selected for further independent development through both contract and grant-supported projects within the Division of Cancer Cause and Prevention. Extension of the Biology Branch Program on in vitro cell transformation promises to further yield practical short-term methods to identify chemical carcinogens and a rational basis for their definition. These methods, as developed in this Branch, have become a major tool in the development of a screening effort in environmental cancer prevention. Members of the Tumor Antigen Section have found that animals harboring tumor cells at a stage before a palpable mass is detectable have defective chemotactic activity. A similar defect has been observed in patients with cancer at a relatively early stage in the disease. In the Immunochemistry Section it has been found that tumor cells, usually refractory to the action of antibody and complement, become susceptible to killing by these immunologic reagents after interaction with chemotherapeutic agents and other metabolic inhibitors. This observation may provide a rationale for the combination of immunotherapy and chemotherapy in the treatment of early cancer. In collaboration with members of the Division of Cancer

Biology and Diagnosis a clinical protocol has been designed and approved that documents an immunologic method, based on a guinea pig model developed in the Cellular Immunity Section, that will be used in attempts to eliminate primary melanomas in humans.

Cytogenetics and Cytology Section - *"Studies (1) carcinogenesis in mammalian cells in vitro and in host-mediate in vivo-in vitro models; (2) cell surface, mutational, chromosomal, and other somatic cell alterations associated with the transition from the growth controlled to the neoplastic state; and (3) effects of potential environmental carcinogens in cells in culture and in intact animals."* As part of the program to determine the factors that influence chemical transformation, studies have been continued on the interaction of potent chemical carcinogens with either weak chemical carcinogens or physical agents. Additional experiments were carried out with a combination of X-irradiation and chemical carcinogen. No transformation occurred as a result of irradiation alone, but transformation by a potent carcinogen was enhanced by pretreatment of the cells with X-irradiation. Irradiation alone had no recognizable effect on cell cycle or on cell cycle or on chromosomes; enhancement may be due to secondary genetic or epigenetic alterations following radiation. Whereas X-irradiation and alkylating chemicals enhance transformation if administered to cell cultures before a potent carcinogen, caffeine, a nontransforming, nonlethal agent, potentiates both toxicity and transformation by chemical carcinogens only when given after the carcinogen. Enhancement depends upon (1) concentration of caffeine, (2) time of addition of caffeine, (3) length of exposure, and (4) carcinogen used. Toxicity increased with caffeine concentration and length of exposure to carcinogen-containing medium. When 0.5-200 µg caffeine/ml medium was added 1 hour after the carcinogen acetoxy-acetylaminofluorene (AcAAF), cloning efficiency decreased and the average number of transformations increased with caffeine concentration. The addition of a constant amount of caffeine (50 µg/ml) for 48 hrs at several intervals after carcinogen resulted in maximum enhancement (10-17-fold) when caffeine was added 4 hrs after carcinogen. Experiments with low concentrations of caffeine permitted no conclusions about the dependency of toxicity upon the number of cells actually being damaged during the S phase, particularly when results with the carcinogens AcAAF and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) were compared. In the former case, cell survival increased when caffeine was added more than 4 hrs after AcAAF, while with MNNG damage was biphasic as indicated by a decrease in cloning efficiency, when caffeine was added even 1 day after carcinogen.

The new cytogenetic banding techniques for identifying mammalian chromosomes have been applied to studies on transformation produced by carcinogenic SA7 virus. This is of particular interest because of immunological evidence that the virus has added new genetic material to the transformed cells. Ten different transformed clones that resulted from transformation by SA7 or by pretreatment with 3-methylcholanthrene or 7,-12-dimethylbenz(a)anthracene followed by SA7 were examined. All transformed clones produced tumors

and all of the different clones including those derived from the combined action of chemical carcinogen and SA7 possessed the SA7 T antigen. Some transformed clones were diploid while others were aneuploid; some had abnormal chromosomes, others did not. Cells treated with chemicals possessed no markers not observed in cells transformed by virus alone. Chromosomal aberrations associated with transformation by carcinogenic viruses were not the same for each clone; this variation was also noted for cells transformed by chemicals. It is concluded, therefore, that neither a common marker nor a specific numerical deviation of chromosomes characterized transformation from either SA7 only or from carcinogen treatment followed by SA7.

Investigations aimed at obtaining transformation of human cells with chemical carcinogens have been continued. Toxicity, morphologic alterations in cell morphology and extension of life span of carcinogen-treated human fetal cells have been observed. In addition, approximately one cell per 10^6 cells from carcinogen-treated cultures will form a progressively growing colony in soft agar. To date, however, no human fetal cells derived from colonies in soft agar have produced a tumor when implanted into a nude mouse (6-12 months observation). Ordinarily, 90% of the hamster or guinea pig transformed cell lines produce tumors in this period. Unlike hamster and guinea pig cells from agar-derived colonies, however, the human cells do not have an infinite life span. Due to the moratorium imposed during 1974 on the experimental use of human fetal cells, no further investigations on human fetal cells were performed. In place of the fetal cells, fibroblast-like cell cultures derived from biopsies obtained from pediatric and adult individuals with the disorder, xeroderma pigmentosum, were treated with carcinogen. Xeroderma cells have been treated with a combination of physical and chemical carcinogenic insults: 250R X-irradiation followed by carcinogens, causing either excision or strand type DNA defects. Morphological alterations have been observed in these cultures which are being evaluated for formation of cell colonies in agar and neoplastic potential.

Investigations are continuing on conditions required to attain optimal developments of in vitro transformation of guinea pig cells as well as studies on the recognition of neoplastic transformation. The transformation of guinea pig cells is inductive rather than selective since it has not occurred spontaneously nor have noncarcinogens been effective during 20 months of subculturing. Whether the transformation is direct or indirect, due to viral activation, is unknown. All cultures, whether or not they were treated with a chemical, have intracellular and extracellular virus-like particles after BRdU treatment. The particles have similarities to, as well as differences from, A, B and C particles of other species. Transformation was observed 4-18 months after exposure to carcinogen. In 4 of 12 transformed cultures, the appearance of morphological transformation coincided with the ability of the cells to produce tumors. In 4 others, transformation preceded tumorigenicity by 3-6 months. The remaining 4 transformed cultures continued to be nontumorigenic for 6 or more months after the appearance of transformation when subculturing was discontinued. Thus, morphological transformation of guinea pig cells does not necessarily reflect the neoplastic state. Alterations in plating efficiency, saturation density, population doubling time, and chromosome number also were not

reliable indicators of tumorigenic potential. A correlate that consistently predicted tumorigenicity was the ability of transformed cells to form colonies of more than 100 cells in 0.35% agar. Cultures with an orderly cell growth pattern or with morphology altered but not transformed by chemical carcinogen neither grew as colonies in agar after seeding of 10^7 cells nor as tumors following inoculation of guinea pigs with 10^8 cells. Morphologically transformed nontumorigenic cultures also failed to form colonies in agar. Thus, morphological transformation may coincide with or precede the ability of cells to grow as colonies in agar. Acquisition of the ability to form colonies in agar, however, appears simultaneously with the potential for the cells to produce a tumor in vivo. Leukocytes from unimmunized guinea pigs also distinguished chemical carcinogen induced, neoplastically transformed guinea pig cells from nontumorigenic cells in a colony inhibition assay. Two short-term quantitative assays that correlate with the tumorigenicity of strain-2 guinea pig fetal cells transformed in culture by chemical carcinogens were found: one measures inhibition of colony growth mediated by nonimmune leukocytes and the other measures skin reactivity in unimmunized syngeneic guinea pigs. After 7-9 days of incubation in the presence of nonadherent peritoneal cells with culture medium (PEC), fewer target cell colonies were observed in cultures with tumorigenic than with nontumorigenic cells. The inhibition of tumorigenic cells was dependent on PEC concentration; at 1000:1 PEC to target cell ratio, as much as an 80% reduction in the number of colonies and a 100% decrease in colony size relative to controls was noted. In the second assay, 2 or 5 x 10^6 tumorigenic or nontumorigenic cells were inoculated intradermally into 12-16 week old male unimmunized syngeneic guinea pigs; skin reactivity, in terms of the degree and persistence of induration during the next four days was greater to tumorigenic than to nontumorigenic cells.

Recent work with bacterial and mammalian cell systems has shown that many carcinogens are also mutagens. Because of this parallelism, it is often suggested that chemical carcinogens are carcinogenic because of their mutagenic action. If this were true, double treatments that enhance neoplastic transformation would be expected to enhance point mutation frequencies, or at least to alter them. To test this possibility, it was necessary to develop a quantitative mutagenesis assay that could accommodate sequential treatments. The V-79 Chinese hamster system was modified to obtain a maximum frequency of resistant or mutant colonies. The frequency of resistant colonies was influenced by inoculum size, concentration of selective agent and the time allowed for mutagen-induced changes to be expressed (expression time) before exposing the cells to selection medium. The quantitative assay developed for studies of mutagen interaction and enhancement of mutagenesis has been rigorously applied to the question of what effect a mild treatment with an alkylating agent methyl methanesulfonate (MMS) might have on a subsequent treatment with the strong mutagen and carcinogen AcAAF. MMS was found to have no effect on the AcAAF-induced mutant frequency; various time intervals between MMS and AcAAF treatments were studied (up to 48 hrs). MMS did enhance AcAAF toxicity, showing that lethality and mutagenicity are not closely related. The mutation results differ from the enhancement of transformation caused by prior treatment of Syrian hamster cells. Transformation enhancement was time-dependent and

was maximal at 48 hrs time between treatments. These results strongly suggest that either (1) Chinese hamster cells cannot be used as a model for Syrian hamster embryo cells in the study of mutagenesis-carcinogenic relationships, or that (2) mutagenesis is not the primary mechanism for enhancement of in vitro transformation caused by AcAAF. Diploid Syrian hamster embryo cells are different from (transformed) Chinese hamster V-79 cell line under the conditions of the mutagenesis assay. Therefore, it is impossible to clearly demonstrate the induction of mutants, since all of the initially drug-resistant clones of cells do not survive continued culturing. Mutants of transformed Syrian hamster cells, however, have been induced with a high frequency of chemical carcinogens and mutagens, and have been isolated for further studies. Continued effort is being directed toward the development of a mutation assay employing diploid Syrian hamster cells derived from fresh fetal material.

Cellular Immunity Section - *"Plans and carries out investigations (1) to determine the types of host immune cells are toxic to cancer cells; (2) to elucidate the process by which these host cells destroy tumor cells; (3) to find methods to increase the number of such cells in the host; and (4) to study possible interactions of carcinogens with host immune cells or with the apparatus responsible for the production of host immune cells."*
(1) Requirements for Inhibition of Tumor Growth by Immune Stimulants--Injection of living BCG into established intradermal tumors causes tumor regression and prevents the growth of microscopic axillary lymph node metastases. It has been found that the presence of specific tumor transplantation immunity correlated with successful immunotherapy. Factors which failed to correlate with successful therapy were delayed cutaneous hypersensitivity reactions to tumor cells or tuberculin, number of mycobacteria in axillary lymph nodes, size and histology of axillary lymph nodes. The implication of these studies is that marked inhibition of tumor growth by immune stimulants requires that the tumor possess transplantation protection antigen(s). Studies with murine fibrosarcomas support this conclusion. Four murine fibrosarcomas were evaluated; 2 tumors did not evoke tumor transplantation immunity, 1 tumor evoked modest tumor transplantation immunity, and 1 tumor evoked strong tumor transplantation immunity (tumor #1023). Tumors which evoked tumor transplantation immunity were responsive to intralesional treatment with BCG; tumors which did not evoke tumor transplantation immunity were unresponsive to intralesional treatment with BCG. Systemic administration of E. coli endotoxin or pyran copolymer was effective treatment for the mice with tumor #1023 and not effective for mice with weakly or nonantigenic tumors. Studies with guinea pig fibrosarcomas also support the above conclusion. Three guinea pig fibrosarcomas were studied; each tumor cell line was produced by in vitro interaction of carcinogens and fetal cells. Injection of living BCG into tumors formed from intradermal injection of these fibrosarcoma cells failed to suppress tumor growth. To date, these tumors have not been found to possess tumor specific antigens. Experiments were conducted to identify effects of methods of BCG preparation and storage on antitumor activity. Fresh frozen and freeze-dried Tice BCG possessed equivalent tumor suppressive properties.

(2) Development of Models of Residual Disease Responsive to Immune Stimulants--Intradermal tumors were excised leaving the host with microscopic axillary lymph node metastases. Intradermal injection of a BCG-irradiated tumor cell vaccine had a modest antitumor effect. Injection of BCG by scarification, intradermal, intravascular or intraperitoneal injection failed to eradicate microscopic axillary lymph node metastases. These results suggest that the mode of BCG administration is a critical variable that determines success or failure of therapy. Intramuscular tumors were excised leaving the host with microscopic metastases in lymph nodes and in lungs. Intralesional injection of BCG prior to amputation prolonged host survival.

(3) Eradication of Established Dermal Tumors and Metastases after Transfusion of Cells from Immunized Hosts--Intracardiac transfusion of peritoneal exudate cells from immunized guinea pigs was effective in eradicating established dermal tumors and axillary lymph node metastases. Immunization of syngeneic guinea pigs to tumor specific transplantation antigens was required for effective treatment. Allogeneic or xenogeneic hosts were immunized to tumor antigens; peritoneal cells from these hosts failed to eradicate tumor growth. Lymphoid cells from immunized hosts were extracted to obtain an extract rich in RNA. Peritoneal cells from normal hosts were incubated with RNA rich extracts from immunized hosts and assayed for antitumor activity. No antitumor activity was detected.

(4) Antitumor Activity of Pyran Copolymer and Listeria Monocytogenes--Infection of hosts with Listeria monocytogenes was effective in inhibiting tumor growth. Tumor growth suppression required infection of the host at the tumor site. Listeria monocytogenes was more effective than BCG in inhibiting tumor growth in mice. Systemic administration of pyran was effective in restricting the growth of 2 murine fibrosarcomas. One correlate of tumor inhibition by pyran was the production in the hosts of a population of macrophages with tumoricidal properties.

(5) Studies of Tumor Graft Rejection in Syngeneic Hosts--The regression of an intradermal tumor in inbred hosts was accompanied by destruction of "innocent bystander" cells. This effect was observed in unimmunized hosts and not in the immunized hosts.

(6) Evaluation of Chemotherapeutic Agents--Ten chemotherapeutic drugs were evaluated for antitumor effect in a guinea pig tumor system. Drugs were administered systemically. No antitumor effects were detected.

Immunochemistry Section - *"(1) Conducts studies into the immunochemistry of antibodies, antigens, and the components of complement; (2) elucidates the mechanisms of cytotoxic reactions; and (3) develops and applies immunochemical tools to the detection and enumeration of normal and malignant antigens."* The mechanism of cytotoxic reactions mediated by antibody and complement was further elucidated. Cells of two guinea pig hepatomas, line-1 and line-10, are resistant to killing by antibody (either against normal liver antigens

or hepatoma associated tumor-specific antigens) and guinea pig complement. It was found that resistance could not be ascribed to lack of antigens, lack of binding of antibody or lack of fixation of complement to the cell surface. It was then postulated that resistance to killing may be due to a cellular defense or repair process requiring metabolic activity. It was found that line-1 and line-10 cells normally resistant to killing by antibody and complement were made sensitive by incubating the cells with various metabolic inhibitors and chemotherapeutic drugs. The following inhibitors were found effective in increasing cell susceptibility to killing: actinomycin D, puromycin, mitomycin C, hydroxyurea, adriamycin, azacytidine, methotrexate, 5 FU and 6 MP. The effect of these inhibitors was dependent on concentration, temperature, time and at the concentration of inhibitor used was entirely reversible within a few hours after removal of the inhibitors. The mechanisms whereby these inhibitors increase susceptibility to immune killing are not understood and are currently being investigated. Irrespective of mechanism(s), the beneficial effect of various drugs and metabolic inhibitors in the treatment of cancer might be due, in part, to their ability to increase the susceptibility of tumor cells to killing by antibody and complement. Studies are therefore underway with members of the Cellular Immunity Section to determine whether these agents can kill line-1 and line-10 tumor cells in vivo.

Macromolecular structures on the surfaces of cells are capable of fluid motion. Movement of cell surface antigens can be induced by antibodies. Since the distribution of cell surface antigens is one of the factors that govern cell susceptibility to killing by antibody and complement, a correlation between cell surface mobility and increased susceptibility induced by inhibitors was sought. The results showed that the increase in drug-induced sensitivity did not correlate with movement of antigen. This was deduced from the facts that cell surface movement was inhibited by sodium azide, sodium fluoride, iodoacetate and cytochalasin B, but these agents did not induce increased sensitivity to killing by antibody and complement; actinomycin D and puromycin increased cell sensitivity to killing without affecting cell surface movement; cycloheximide inhibited cell surface movement and increased cell susceptibility.

Since cell surface antigens play a crucial role in initiating attack by immune mechanisms, efforts to isolate and characterize tumor antigen continued. Soluble tumor antigen of line-1 and line-10 cells was isolated from ascitic fluid of animals bearing tumor in the peritoneal cavity. The antigen, identified by its biological and immunochemical properties, has been shown to be excluded by Sephadex G-200, indicating a molecular weight greater than 2×10^5 Daltons. The antigen does not sediment in the ultracentrifuge under conditions where molecules with a molecular weight greater than 8×10^5 would sediment. The data thus indicate that the antigen is soluble and has a molecular weight between 2×10^5 and 10^6 Daltons.

The interaction of cell surface receptors with complement is initiated by appropriate immunoglobulins fixed to cell surface antigens. A hitherto unrecognized factor that prevents the first component of complement from interacting with immunoglobulin attached to antigen has been identified. This factor is produced by normal ("non-stimulated") lymphoid and peritoneal exudate cells. It is capable of binding to the surface of sheep erythrocytes; its presence can be detected by inhibition of complement lysis of the cells, by fixation of activated C1 and destruction of precursor C1. This new factor may represent yet another defense mechanism against the cytotoxic action of complement activity. The factor is now being analyzed for its molecular nature and mode of action.

Tumor Antigen Section - *"(1) Conducts a research program on tumor specific antigens in experimental animals and man (a) development of methods for antigen assay (b) extraction and purification of antigens for induction of immune defense against tumor cells and for production of antibodies that can be used as markers in carcinogenesis studies; (2) investigates problems in cell biology related to host response to tumor antigens (a) cell membrane antigen movement (b) mononuclear cell chemotaxis (c) isolation and mechanism of action of lymphotoxin."* During the past year work in this Section has covered the following three areas:

(1) Purification of Tumor Antigen and Immunogenicity of the Products--Studies on 3MKCl extracts of line-10 hepatoma cells were continued and 2 major points were documented. First, line-10 antigenic activity in the extracts is heterogeneous both in molecular size as measured by gel filtration and in charge as indicated by elution on DEAE. This heterogeneity emphasizes the need for an improved first solubilization step to facilitate structural studies of tumor antigens. Second, there is a remarkably large discrepancy between antigenicity of line-10 KCl extracts (as measured by capacity to elicit a delayed cutaneous hypersensitivity reaction when injected intradermally into line-10 immune guinea pigs) and the capacity of the extract to protect against line-10 tumor challenge. Seven hundred times more antigen in the form of KCl extract was required to equal the protective effect of viable cells. It would be of great interest to know whether the difference between skin reactivity and capacity to protect against tumor challenge is due to an alteration in the quality of the antigen, continued production of antigen by viable cells to make up the 700-fold difference, differences in the mode of presentation to antigen-reactive cells or that skin reactivity and protection are due to separate antigens.

(2) Mechanisms of Tumor Cell Killing--Specific antigen or mitogen-stimulated guinea pig peritoneal exudate (PE) cells were cytotoxic to syngeneic target cells in vitro. This cytotoxicity reaction was immunologically nonspecific in that the target cells were antigenically unrelated to the stimulus which initiated the immune response. In collaboration with the Cytogenetics and Cytology Section, the susceptibility of several tumorigenic and nontumorigenic syngeneic cell lines to this cytotoxic reaction was examined. Cytotoxicity was estimated by measuring the release of ^{3}H-thymidine from pre-labeled

target cells. With optimal concentrations of mitogen and PE cells, all tumorigenic cell lines were more affected than the nontumorigenic cell lines. Nontumorigenic cells had less specific release of radioactive label than the tumorigenic cells. A differential effect of activated macrophages on tumor cells and normal cells was also found in the mouse. Peritoneal macrophages from mice infected with Mycobacterium bovis, strain BCG, were cytotoxic to tumor cells in vivo and in vitro. These activated macrophages affected non-neoplastic cells to a lesser degree.

In collaboration with the Cell Physiology and Oncogenesis Section (Laboratory of Biochemistry, DCBD, NCI), the interaction of BCG-activated macrophages with target cells in vitro was examined in detail. Neoplastic and non-neoplastic cell lines derived from a single cloned syngeneic embryo cell were used. Macrophage-target cell interaction was analyzed by phase-contrast cinemicrography and by release of ^{3}H-thymidine from prelabeled target cells. BCG-activated macrophage-tumor cell interaction was characterized by repeated and relatively short (about 2 hours) cell-to-cell contacts which induced tumor cytostasis and lysis. BCG-activated macrophages were partially cytostatic to non-neoplastic cells but target cell death was not observed. Translational movement (an index of cell motility) of activated macrophages on tumor cell monolayers was up to 5 times greater than that observed on non-neoplastic target cells. Tumoricidal macrophages were present at 3-7 days and persisted for six weeks after a single BCG injection. Impaired response of peripheral blood monocytes to a standard chemotactic stimulus in cancer patients was reported last year. An animal model has been developed for further study of this phenomenon and it has been found that the chemotactic responsiveness of peritoneal macrophages is impaired within 1 week after subcutaneous injection of a transplantable tumor into mice.

The destruction of tumor target cells by supernatants from stimulated spleen cell cultures has been measured by thymidine release from pre-labeled cells. In contrast to published assays of lymphotoxin, this measures cell death rather than the total result of cell death and proliferation inhibition. The factor characterized by the thymidine release assay has a molecular weight around 50,000 and an isoelectric focusing range of 4.5-5.0. This is very different from the characteristics of mouse lymphotoxin reported previously (molecular weight in the range of 80-150,000 and highly alkaline isoelectric point).

(3) Chemotaxis--Since inflammatory cells may be attracted to a tumor destruction site by chemotactic mechanisms, a study has been undertaken of chemotaxis mechanisms including the production of chemotactic stimuli and the effect of various modulators of chemotactic response. Agents which increase mouse macrophage tumoricidal capacity were found to increase macrophage chemotactic responsiveness. Agents which induce an influx of cells into the peritoneal cavity without inducing macrophage activation (oil, starch) do not increase chemotactic responsiveness.

Under certain conditions, sites of delayed cutaneous hypersensitivity reactions have large accumulations of basophils. This is in contrast to the tuberculin reaction in which the predominant inflammatory cell is the macrophage. To elucidate the chemical basis of the difference in cell infiltrates, mitogen- or antigen-induced lymphokines have been analyzed for human basophil or monocyte chemotactic activity. The two activities are remarkably similar, showing, for example, 2 distinct peaks on DEAE chromatography, both of which have both monocyte and basophil chemotactic activity. One fact favoring a chemical difference in the 2 activities is that basophil chemotactic factor is consistently less stable than the monocyte factor. It was shown last year that supernatants from stimulated lymphocyte cultures greatly increase the responsiveness of human basophils to complement derived chemotactic factor. Recent experiments show that this augmentation factor is chemically distinct from lymphocyte-derived basophil chemotactic factor.

Project No. ZO1-CP-04664-05-BGY
1. Biology Branch, Carcinogenesis, DCCP
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunologic Approaches to the Prevention and Treatment of Cancer

Previous Serial Number: Same

Principal Investigators: Berton Zbar, M.D. and Herbert J. Rapp, Sc.D.

Other Investigators: Robert C. Bast Jr., M.D., Richard Harmel Jr., M.D. and Takeshi Ogura, M.D.

Cooperating Units: For supply of BCG and studies of different BCG preparations: Trudeau Institute, Saranac Lake, New York. For evaluation of combinations of surgery and immunotherapy: Howard Smith, M.D. and Steven Rosenberg, M.D., Surgery Branch, DCT, NCI. For studies of MER and BCG-SS: Percy Minden, National Jewish Hospital, Denver, Colorado. For studies of in vitro transformed cells: Charles Evans, M.D., Cytogenetics and Cytology Section, Biology Branch, CG, DCCP, NCI.

Man Years:

Total	3.0
Professional	2.0
Other	1.0

Project Description

Objectives:

The primary objective of this project is to establish conditions and methods suited to the prevention and treatment of cancer. This objective is based on the finding that tumors contain unique antigens capable of eliciting the formation of tumor specific humoral antibodies and of causing the appearance of cells which react specifically with tumor antigens. Transplantable syngeneic guinea pig tumors will be used to investigate conditions for effective immunotherapy.

Methods Employed:

Tumors. Malignant hepatomas have been induced in strain-2 guinea pigs by feeding them diethylnitrosamine in their drinking water. Transplantable ascites tumors have been derived from primary hepatomas. Two of these ascites variants are being maintained by periodic intraperitoneal transplantation. Portions of these tumors are periodically frozen and stored in liquid nitrogen.

Studies on effect of method of preparation on tumor suppressive properties of BCG, and treatment of microscopic lymph node metastases were carried out with cells of a transplantable hepatoma (line-10). This tumor when injected into the skin produces a progressively growing papule and regional lymph node metastases within a few days.

Tumor suppressive properties of mycobacterial preparations. Tumor cells were injected intradermally into unimmunized strain-2 guinea pigs. When established intradermal tumors and regional lymph node metastases were present (7 days after intradermal injection) BCG preparations were injected into the tumor.

Effect of method of preparation on tumor suppressive properties of BCG. Freeze dried preparations of the Tice substrain of BCG were obtained from the Tuberculosis Research Laboratory of the University of Illinois. BCG cultivated in liquid medium in the absence of a detergent grew as a pellicle on the surface of the medium. Pellicles were ground in a mechanical mill; the dispersed preparation was frozen and dried. About 5% of the bacteria in these preparations were viable.

Fresh frozen preparations of the Tice substrain of BCG were prepared at the Trudeau Institute. BCG cultivated in the presence of a detergent grew below the surface of the medium as a dispersed culture. The bacteria in culture medium were frozen. About 60-90% of bacteria in these preparations were viable.

Microscopic axillary lymph node metastases. One million line-10 cells were injected intradermally into strain-2 male guinea pigs. Seven days after inoculation, intradermal tumors were excised. Guinea pigs treated in this manner were free of intradermal tumors but had 10^5 tumor cells in the superficial distal axillary lymph node.

Major Findings:

Conditions required for eradication of microscopic axillary lymph node metastases:

1. BCG administered by scarification, by intradermal, intravenous, intraarterial, or intranodal injection was ineffective in eliminating residual malignant disease.

2. Injection of BCG into established intradermal tumors prior to local excision was successful in eradicating microscopic axillary lymph node metastases

3. Excision of the dermal tumor 20 minutes, or 1 day after intratumor injection of BCG prolonged survival but did not cure a significant number of animals. Excision of the dermal tumors 7 days after intratumor injection of BCG cured a significant number of animals.

4. Several factors were evaluated as possible correlates of successful immunotherapy. The development of tuberculin hypersensitivity, the magnitude of regional adenopathy and the number of BCG in axillary nodes were not useful correlates.

5. Histologically, the presence of tumor cells, multiple focal granuloma, or of histiocytosis in axillary nodes failed to correlate with results of therapy.

6. The development of specific tumor transplantation immunity provided the best correlate of successful immunotherapy.

Antitumor activity of MER and of a saline extract of BCG in National Institutes of Health and National Jewish Hospital strain-2 guinea pigs:

1. MER injected in admixture with tumor cells suppressed tumor growth in 50 percent of NIH strain-2 guinea pigs.

2. MER injected into established intradermal tumors caused tumor regression and prevented the growth of microscopic lymph node metastases in 13 percent of NIH strain-2 guinea pigs.

3. BCG, MER or BCG-SS injected into NIH strain-2 guinea pigs prior to injection of tumor cells failed to prevent tumor growth in NIH strain-2 guinea pigs.

4. There was some evidence that MER or BCG-SS prevented tumor growth in National Jewish Hospital strain-2 guinea pigs.

Susceptibility of tumors to antitumor activity of BCG: tumors derived from strain-2 cells transformed in vitro:

1. Two tumor lines were studied: 104C1 and HM4C1 Both tumor lines grew progressively after intradermal injection in strain-2 guinea pigs. The threshold for intradermal growth was: tumor 104C1, about 2 x 10^3 cells; tumor HM4, about 2 x 10^4 cells.

2. Injection of living BCG into established 104C1 dermal tumors failed to lead to tumor regression. Intradermal injection of living BCG in admixture with 10^6 or 10^5 104C1 cells failed to suppress tumor growth. By transplantation protection tests, tumor 104C1 was not antigenic.

3. Injection of living BCG into established HM4C1 dermal tumors failed to lead to tumor regression.

Susceptibility of tumors to antitumor activity of BCG: methylcholanthrene induced sarcomas in C3H/HeN mice:

1. Four tumor lines were studied: 1023, 1037, 1038 and 1063. Mice immunized with tumor #1023 were able to reject a dermal challenge of 5×10^6 cells; mice immunized with tumor #1037 were able to reject a dermal challenge of 5×10^5 cells; mice immunized with tumor #1038 or #1063 were unable to reject a dermal challenge of tumor cells.

2. Susceptibility of tumors to the antitumor activity of BCG correlated with antigenicity as determined by transplantation tests.

Antitumor activity of BCG-tumor cell vaccines:

1. Intradermal injection of a BCG-tumor cell vaccine led to inhibition of growth of microscopic axillary lymph node metastases.

Comparison of antitumor activity of fresh frozen and freeze dried Tice BCG:

1. Fresh frozen and freeze dried Tice BCG possessed equivalent tumor suppressive properties.

Significance to Biomedical Research and the Program of the Institute:

BCG preparations are potent inhibitors of tumor cell growth. Although this observation has been repeatedly made, it has been difficult to harness the tumor suppressive properties of BCG. Studies on the conditions required for eradication of microscopic axillary lymph node metastases suggest that certain variables are essential for a successful clinical trial of BCG. These studies suggest the need for a carefully designed trial of efficacy of intralesional BCG and surgery in the treatment of patients with primary cutaneous malignant melanoma and microscopic metastases. Such a trial is to be conducted by members of the Surgery Branch, DCBD.

Proposed Course of Project:

To continue to perform experiments designed to determine requirements for successful administration of BCG. Emphasis will be placed on critical temporal aspects of BCG and tumor cell administration, on conditions for optimal use of BCG-tumor cell vaccines and on antitumor activity of other immunopotentiators.

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Project No. Z01-CP-04665-05-BGY
1. Biology Branch, Carcinogenesis, DCCP
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mechanism of Delayed Hypersensitivity and Tumor Graft Rejection

Previous Serial Number: Same

Principal Investigators: Berton Zbar, M.D., and Herbert J. Rapp, Sc.D.

Other Investigators: Robert C. Bast Jr., M.D., Richard Harmel Jr., M.D.

Cooperating Units: For studies of transfer of tumor immunity: Howard Smith, Surgery Branch, DCBD. For studies with immune RNA: Yosef Pilch, University of California at Los Angeles.

Man Years:

Total	2.2
Professional	1.2
Other	1.0

Project Description

Objectives:

The main objectives of this project are: 1) to define the mechanism of the immunologic rejection of antigenic tumors and 2) to determine the basis for suppression of tumor growth at the site of bacterial infections.

Methods Employed:

Guinea pig tumors. Malignant hepatomas have been induced in strain-2 guinea pigs by feeding them diethylnitrosamine in their drinking water. Transplantable ascites and intramuscular variants of these tumors currently maintained in this laboratory are used.

Systemic transfer of tumor graft rejection. Animals were immunized to line-1 tumor, to line-10 or to BCG. Peritoneal exudate cells were obtained after intraperitoneal injection of sterile mineral oil. The peritoneal exudate cells were washed and injected into the hearts of guinea pigs bearing established intradermal tumors.

Transfer of tumor immunity with extracts rich in RNA. Spleens and lymph nodes were removed from strain-2 guinea pigs immunized to line-10 and BCG. RNA was extracted from these tissues by the hot phenol method (Pilch). Extracts were characterized by chemical analysis of RNA, DNA and protein and by ultracentrifugation on sucrose density gradients. Extracts were incubated with lymphoid cells from normal animals. Cells treated with RNA were tested for ability to respond to tumor antigen in vitro, (by the macrophage migration inhibition assay) and in vivo, (by a tumor suppression assay). In addition, RNA-rich extracts together with dextran sulfate were injected directly into the skin or footpads of normal guinea pigs; these animals were subsequently challenged with an intradermal inoculation of tumor cells.

Major Findings:

Systemic transfer of tumor graft rejection.

1. Peritoneal exudate cells were obtained from strain-2 guinea pigs immunized to line-10 and injected into the hearts of guinea pigs bearing established dermal line-10 tumors. This procedure led to regression of the dermal tumors and prevention of growth of lymph node metastases.

2. One hundred million peritoneal exudate cells were required for regression of the line-10 dermal tumors.

3. Peritoneal exudate cells obtained from strain-2 guinea pigs immunized to line-1 or BCG failed to cause regression of line-10 dermal tumors.

4. Peritoneal exudate cells obtained from strain-13 guinea pigs immunized to line-10, and peritoneal exudate cells obtained from sheep immunized to line-10 failed to cause regression of line-10 dermal tumors.

5. Intralesional injection of BCG improved the antitumor effect of intracardiac injection of peritoneal exudate cells.

6. Peritoneal exudate cells were obtained from strain-2 guinea pigs immunized to line-10 and injected into the hearts of guinea pigs with microscopic axillary lymph node metastases. This procedure prevented the growth of microscopic axillary lymph node metastases.

RNA transfer of tumor graft rejection.

1. Attempts were made to transfer immunological responses with subcellular extracts of lymphoid cells. Subcellular extracts rich in RNA converted peritoneal exudate cells obtained from normal guinea pigs to cells responsive to line-10 tumor antigen (macrophage migration inhibition assay). The degree of reactivity of converted peritoneal exudate cells was less than the degree of reactivity of cells from immune donors.

2. Peritoneal cells treated with "immune" RNA were injected into the hearts of guinea pigs. This procedure did not prevent tumor growth.

3. RNA rich extracts with dextran sulfate were injected intradermally or into the footpads. This procedure did not prevent tumor growth.

Significance to Biomedical Research and the Program of the Institute:

There is considerable interest in the possible use of immune RNA as an antitumor agent. Systemic transfer of tumor graft rejection with intact lymphoid cells provides a model for studies of transfer of graft rejection with subcellular extracts. Although to date such studies have been negative in our laboratory, the animal model developed should permit a definitive answer to the question within 1-2 years.

Proposed Course of Project:

To continue attempts to inhibit tumor growth in vitro with cellular extracts rich in RNA. Cellular extracts rich in RNA will be obtained from Drs. Dray and Pilch and tested for ability to transfer resistance to tumor growth.

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Zwilling, B. S., Leonard, E. J., Bast, R. C., Zbar, B.: Destruction of syngeneic tumors by tuberculin stimulated peritoneal exudate cells from guinea pigs immunized to Mycobacterium bovis, strain (BCG). J. Natl. Cancer Inst. 53: 541-546, 1974.

Project No. Z01-CP-04679-03-BGY
1. Biology Branch, Carcinogenesis, DCCP
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Interaction of Carcinogenic Polycyclic Hydrocarbons with Mammalian Lymphoid Tissue

Previous Serial Number: Same

Principal Investigators: Robert C. Bast, Jr., M.D., Harry V. Gelboin, Ph.D.
Herbert J. Rapp, Sc.D.

Other Investigators: Takao Okuda, Ph.D.

Cooperating Units: Chemistry Branch, CG, DCCP, NCI

Man Years:

Total	: 1.5
Professional	: 1.0
Other	: 0.5

Project Description

Objectives:

The main objectives of this project are: 1) to measure the effect of polycyclic hydrocarbons on the microsomal enzymes of macrophages and lymphocytes; 2) to develop a dependable, convenient in vitro assay for aryl-hydrocarbon hydroxylase (AHH) in human peripheral blood monocytes; and 3) to find whether interaction of polycyclic hydrocarbons with macrophages facilitates the induction and/or expression of contact hypersensitivity to these compounds.

Methods Employed:

AHH activity of human peripheral blood monocytes. Mononuclear leukocytes were obtained from heparinized whole blood by centrifugation on a discontinuous Ficoll-Hypaque gradient. Monocytes adhered to plastic culture dishes and could be separated from non-adherent lymphocytes. After incubation for 18 hours medium containing or lacking the polycyclic hydrocarbon benz(a)-anthracene (BA) was added. Twenty-four hours later monocytes were scraped from culture dishes, counted and assayed for AHH.

AHH activity of guinea pig lymphoid tissue. Inbred strain-2 or strain-13 guinea pigs received methylcholanthrene (MCA) in corn oil, or corn oil alone, intradermally in the footpads. After 24 hours regional lymph nodes and spleen were excised and assayed for AHH activity and for protein content.

Induction and elicitation of cutaneous hyperreactivity to DMBA in mice. Dimethylbenz(a)anthracene (DMBA) in acetone, or acetone alone, was applied to the backs of C57Bl/6J and DBA/2J mice. One or two weeks later DMBA or acetone was painted on the ears of the mice, and ear thickness was measured at different intervals thereafter. Similar experiments were conducted with the contact allergen dinitrochlorobenzene (DNCB).

Major Findings:

1. AHH activity is present in human peripheral blood monocytes and is increased by treatment with BA in cell culture. An assay has been developed that will permit studies of AHH in monocytes from cancer patients and from control populations.

2. AHH activity of human peripheral blood monocytes requires NADPH and is inhibited by carbon monoxide and by 7,8 benzoflavone. The BA-induced increase of AHH activity can be inhibited by cycloheximide and by actinomycin D.

3. AHH activity of guinea pig lymphoid tissue was increased by footpad injection of MCA. There was no consistent difference in response between strain-2 and strain-13 guinea pigs.

4. C57Bl/6J mice developed specific cutaneous hyperreactivity to DMBA; DBA/2J mice did not. Increase in ear thickness was maximal 8-10 days after application of the DMBA. Both strains could be sensitized with DNCB. Challenge reactions were maximal 2-4 days after application of DNCB.

Significance to Biomedical Research and the Program of the Institute:

1. The microsomal mixed function oxygenase complex of mammalian cells metabolizes a variety of carcinogenic polycyclic hydrocarbons and facilitates their binding to cellular protein and DNA. AHH, a part of this complex, detoxifies several polycyclic hydrocarbons, but can also catalyze their conversion to more carcinogenic forms.

Studies of AHH in humans have been severely limited by the lack of a convenient assay for the enzyme in a readily available tissue. Newly developed assays depend upon the transformation of human peripheral blood lymphocytes into lymphoblasts in the presence of PHA or pokeweed mitogen. PHA responsiveness can vary dramatically in different disease states and the ability of experimental animals to respond to PHA is determined genetically. Development of an assay for AHH in monocytes that does not require mitogens may avoid these difficulties.

2. Cutaneous application of polycyclic hydrocarbon carcinogens can produce immunologically specific contact hypersensitivity. These low molecular weight hydrocarbons probably attain immunogenicity by conjugation with host proteins. Microsomal enzymes might contribute to the formation of immunogenic conjugates by converting non-reactive parent compounds to reactive intermediates capable of binding covalently to cellular macromolecules. Genetic analysis of AHH levels and of immunologic hyperreactivity to polycyclic hydrocarbons in different mouse strains may provide evidence to test this hypothesis.

Proposed Course of Project:

1. To study AHH levels in monocytes from patients with lung cancer and in control populations.

2. To elucidate the processes controlling induction of AHH and metabolism of polycyclic hydrocarbons in human peripheral blood monocytes.

3. To explore the possible role of AHH in processing polycyclic hydrocarbons for participation in contact hypersensitivity.

Publications

Bast, R. C., Jr., Whitlock, J. P., Jr., Miller, H., Rapp, H. J., Gelboin, H. V.: Aryl hydrocarbon (benzo(a)pyrene) hydroxylase in human peripheral blood monocytes. Nature 250: 664-665, 1974.

Project No. ZO1-CP-04575-01-BGY
1. Biology Branch, Carcinogenesis, DCCP.
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Relationships Between In Vitro Mutagenesis and Chemical Carcinogenesis.

Previous Serial Number: None

Principal Investigator: Brian C. Myhr, Ph.D.

Other Investigator: Joseph A. DiPaolo, Ph.D.

Cooperating Units: None

Man Years:

Total	: 1.6
Professional	: 1.1
Other	: 0.5

Project Description

Objectives:

The ultimate objective of this project is to determine whether mutational processes are involved in the neoplastic transformation of mammalian cells by diverse chemical and physical mutagens. The accomplishment of this goal depends on quantitative assays for mutant frequencies and the ability to perform both mutagenesis and carcinogenesis assays with the same type of cultured cells. Accordingly, the current objectives are:

1. To determine whether mutant frequencies in a Chinese hamster cell line are enhanced by combinations of chemical treatments or by the action of inhibitors of DNA repair under conditions known to result in enhancement of neoplastic transformation of Syrian hamster embryo cells.

2. To quantitatively detect mutants in cultures of normal Syrian hamster embryo cells treated with chemicals that are both mutagenic and carcinogenic.

Methods Employed:

Chinese hamster cells (V79-4) have been utilized by other investigators to demonstrate the emergence of cells that survive in the presence of azaguanine or thioguanine after the cell population has been treated with known mutagens. The basis for survival is the lack of incorporation of these drugs into the

cellular DNA. This cellular change appears to be a mutation, since the loss or reduced levels of a functional enzyme, hypoxanthine guanine phosphoribosyl transferase (HGPRT), can usually be demonstrated.

The drug-resistance marker is being used in the quantitative mutation assay procedure developed in this laboratory for Chinese hamster cells. Methods are continuously being changed in attempts to demonstrate drug-resistant cells in treated Syrian hamster embryo cell cultures. Mutagenic treatments correspond to those used previously in studies of malignant transformation of Syrian hamster cells; the mutagens include methylmethane sulfonate (MMS), N-acetoxy-2-fluorenylacetamide (AFAA), and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

Major Findings:

1. Treatment of Chinese hamster V79-4 cells with 500uM MMS, a weak carcinogen, has been shown to have no effect on the mutant frequency induced by a second treatment with 7uM AFAA, a strong mutagen and carcinogen. Various time intervals between MMS and AFAA treatments (up to 48 hrs) have the same result. MMS did enhance AFAA toxicity, proving that lethality and mutagenicity are separable phenomena. The mutation results differ from the enhancement of malignant transformation caused by prior MMS treatment of Syrian hamster cells. This enhancement was time depenoent and was maximal (about 8-fold) with 48 hrs between treatments.

2. Caffeine is an inhibitor of DNA repair and published reports of its effects on chemical mutagenesis are in conflict with claims for both enhancement and inhibition of mutagenic efficiency. It was found that 50ug/ml caffeine added 1 hr or 2 hrs after AFAA treatment of Chinese hamster cells has no effect on AFAA mutagenesis. In contrast, 50ug/ml caffeine enhances AFAA-induced transformation of Syrian hamster cells; the enhancement is time dependent and is maximal (about 8-fold) when caffeine is added at 4 hrs after AFAA. Studies are in progress with different concentrations of caffeine and times of addition after the initial mutagenic insult.

3. Diploid Syrian hamster embryo cells are different from (transformed) Chinese hamster V79 cell line under the conditions of the mutagenesis assay. Thus far it is impossible to clearly demonstrate the induction of mutants, since all of the initially drug-resistant clones of cells do not survive continued culturing. Mutants of transformed Syrian hamster cells, however, have been induced with a high frequency by chemical carcinogens and mutagens and have been isolated for further studies. Continued effort is being directed toward the development of a mutation assay employing diploid Syrian hamster cells derived from fresh fetal material.

4. Cell lines are often infected with Mycoplasma organisms without the knowledge of the investigator. It was shown that the appearance of chemically-induced mutants is greatly delayed in time when a Chinese hamster cell line injected with M. hyorhinis is used. Therefore, it becomes important to demonstrate that cell lines used for the induction of drug-resistant mutants are Mycoplasma-free.

Significance to Biomedical Research and the Program of the Institute:

Mutagenesis has long been a suspected, but undemonstrated, mechanism for chemical and physical carcinogenesis. Since 80% of human cancers have been estimated to be caused by chemicals, it becomes extremely important to understand the principal mechanisms involved. The ability to perform, simultaneously, mutation and malignant transformation assays with a cell system exposed to mutagens/carcinogens is a crucial step toward understanding the mode of action of these chemicals.

Proposed Course of Project:

A continued effort will be made to demonstrate mutagenesis in Syrian hamster embryo cell cultures. This may include transplacental mutagenesis and the choice of another genetic marker. The assay must then be placed on a quantitative basis to be able to compare mutant frequencies and study enhancement conditions. The Chinese hamster cell line will also be used for further studies of the mutation process itself and the mutagenic efficiencies of several activated carcinogens. The study should also be extended to existing man-mouse cell hybrids to demonstrate mutagenesis of specific human chromosomes.

Publications

Myhr, B. C. and DiPaolo, J. A.: Requirement for cell dispersion prior to selection of induced azaguanine-resistant colonies of Chinese hamster cells. Genetics (In Press).

Project No. Z01-CP-04629-10-BGY
1. Biology Branch, Carcinogenesis, DCCP.
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Mechanism of Cell Transformation

Previous Serial Number: Same

Principal Investigator: Joseph A. DiPaolo, Ph.D.

Other Investigators: Paul J. Donovan, B.S., Charles H. Evans, M.D., Ph.D., Robert C. Gallo, M.D. and Nicolae C. Popescu, Ph.D.

Cooperating Units: Laboratory of Tumor Cell Biology, DCT.

Man Years:

Total	: 4.0
Professional	: 2.5
Other	: 1.5

Project Description

Objectives:

The primary objective of this project is to establish conditions and methods for in vitro quantitative study of chemical transformation. The immediate targets of study are:

1. Development of rapid assay systems for chemical carcinogens suitable for the screening of compounds and populations.

2. Define the required conditions for the transformation of human fetal cells.

3. Determination of ways to increase the susceptibility of primary cell lines or cell strains to chemical transformation.

4. Development of assays to determine whether in vitro transformation is accompanied by the appearance of tumor specific antigens.

5. Determination of the role of nononcogenic and oncogenic viruses in chemical transformation.

6. Determination of the ultrastructural changes of cells during the course of in vitro chemical transformation so that they may be contrasted with normal cells and with information pertaining to viral transformation.

7. Determination of the role of radiation and alkylating compounds in enhancing neoplastic transformation by chemicals.

8. The transformation of epithelial-like cells which result in the formation of carcinomas when the cells are transplanted into animals.

9. Analysis of variations in populations of somatic cells and their correlation with genetic changes.

Methods Employed:

All procedures performed are with the view of quantitative phenomena in vitro. Such procedures are required in order to determine whether or not the transformation observed is due to the direct or indirect effect of the carcinogen and in order to study the early events associated with in vitro transformation. Cells used come from freshly isolated cells from animals and humans that as controls have many of the attributes of "normal" cells and from cell lines which are known to exhibit some of the properties associated with non-transformed cells. Discrete cells are grown in complete medium in the presence or absence of irradiated rat or hamster cells. The cells may be derived from whole embryos or may be from specific organs. The cells are exposed to chemical carcinogen transplacentally or prior to or subsequent to seeding the cells in Petri dishes.

Approximately one week subsequent to treatment the cells are examined under phase or under stained conditions for number of transformed colonies, toxicity and spectrum of morphology of both the normal and transformed colonies. The frequency of transformation is expressed in a number of different ways. These take into consideration the observed rate of transformation on a per-cell basis or on a number of colonies obtained.

Major Findings:

Whereas treatment of cells by X-irradiation does not result in transformation of Syrian hamster cells, the use of ultraviolet irradiation does. UV (2537Å) sensitivity on colony forming ability resulted in a D_0 of 58 ergs/mm^2. UV produced 1, 1.7 and 2.6 T/dish with 15, 30 and 45 ergs/mm^2, respectively. When cells seeded to form colonies were treated with UV followed by benzo(a)-pyrene or N-acetoxy-fluorenylacetamide commencing 5 min to 72 hrs post UV, the difference in T incidence from chemical only was not statistically significant. These results are in contrast to the enhancement obtained from the addition of a number of chemical carcinogens to cells pretreated with X-rays or methylmethane sulfonate. When mass cultures were X-rayed with 250R, seeded for colony formation and treated with 15 or 30 ergs/mm^2 of UV 48 hrs later, the enhancement of UV T was 12X and 5X on a colony basis and 6X and 3X on a dish basis, respectively. These data appear to indicate that enhancement of transformation follows X-ray type of insult.

Recently, it has been reported that the addition of some chemicals to living cells results in identical chromosomal bands without the requirement for a

special post-fixation treatment. We found that some chemical carcinogens produced chromosome bands in human and hamster cells in culture while noncarcinogens did not. With the concentrations used, 7,12-dimethybenz(a)anthracene (DMBA), benzo(a)pyrene (BP), aflatoxin B_1, N-acetoxy-1-fluorenylacetamide, N-methyl-N'-nitro-N-nitrosoguanidine, and 4-nitroquinoline - oxide, but not urethane, produced bands in chromosomes of living human and hamster cells; pyrene, phenanthrene, perylene noncarcinogenic chemicals were ineffective in producing bands. The crossbands occurred only after 24 hrs treatment, while 4 or 8 hrs of treatment with the same concentrations failed to produce chromosome bands. The incidence of metaphases with chromosomes exhibiting bands varied with the carcinogen and in general neither human leukocytes nor Syrian hamster cells was more susceptible although the highest incidence of banded metaphases, 80%, occurred when DMBA was applied to human leukocytes. The immediate effect of the carcinogens in both human and hamster cells was a partial inhibition of the cell multiplication, except for urethane which seemed to stimulate mitosis. Noncarcinogens had little or no effect on cell division. In these experiments, breaks and rearrangements occurred following carcinogen treatment which inhibited cell multiplication. Examination of chromosomes of cells transformed by chemical carcinogens reveals that the changes in number of chromosomes or new marker chromosomes as indicated by banding patterns are not usually related to the process of carcinogenesis but are reflections of secondary alterations. Therefore, the manifestations reported here are not related to transformation nor can they be expected to serve as an accurate index for surveying compounds for possible carcinogenicity.

The host-mediated concept has also been utilized with guinea pig fetuses exposed in utero. With this species, morphological alteration was seen shortly after culturing the cells but bonafide transformation did not occur for several months. Nor did it necessarily appear with the ability of transformed cells to grow as tumors in irradiated syngeneic newborn guinea pigs. Formation of colonies in agar appeared subsequent to or concomitantly with morphologic transformation and in every case developed concurrently with the potential for neoplastic growth. The use of guinea pigs for the study of identification of potential carcinogenic activity of chemical systems and for the study of the mechanisms of carcinogenesis is the subject of a separate report.

One of the major concerns of this laboratory has been the role of chromosome changes in the initiation of neoplastic transformation with quantitative tissue culture procedures. The transformation produced by carcinogenic viruses is of particular interest because of immunological evidence that new genetic material has been added to the cells. In the present study, both conventional chromosome techniques and the Giemsa banding technique were used to analyze seven different hamster cell clones transformed by the carcinogenic Simian virus SA7 and three clones obtained by pretreatment with chemical carcinogens that affect stability of cellular DNA and thus increase the susceptibility of cells to transformation by the SA7 virus. All isolated clones, whether treated with chemical carcinogen and SA7 or with SA7 only, were morphologically typical of SA7 transformation and contained viral-specific SA7 T antigen. Cells from all the transformations produced fibrosarcomas in newborn or weanling hamster within three weeks when 10^6 cells were injected subcutaneously. In

terms of numerical changes and new chromosomes, the chromosomal characteristics of these transformed cells are consistent with the results we have previously published for chemical carcinogens. Chromosome constitution of different clones varied; a normal diploid or an aneuploid constitution with minor structural or numerical deviation was found in some, while in others aneuploidy was associated with increased numerical changes as well as with abnormal chromosomes which are considered as markers. No consistent specific chromosome changes characterized transformation from SA7 or carcinogen pretreatment between the different pools of cells used. Although a lack of consistent chromosome pattern was associated with SA7 viral transformation, the possibility remains that extensive invisible minor genetic changes may have occurred. The development of transformation is usually not associated with or due to visible karyotypic abnormalities though it is possible that some cancer may develop such cytogenetic aberrations concomitant with or as a result of a neoplastic state.

Caffeine, at the concentrations used, is nontransforming and nonlethal to cultured Syrian hamster cells. While a number of agents will enhance transformation if given before a potent carcinogen, caffeine was effective only if given after the known carcinogen. Enhancement was obtained with benzo(a)pyrene, N-acetoxy-2-fluorenylacetamide (AcAAF), or N-methyl-N'-nitro-N-nitrosoguanidine. This enhancement depends upon: (a) concentration of caffeine; (b) time of addition of caffeine; (c) length of exposure; and (d) carcinogen used. The addition of caffeine to cells treated 1 hr previously with carcinogen resulted in the potentiation of toxicity. Toxicity increased with caffeine concentration and length of exposure to carcinogen containing medium. With 50µg caffeine per ml medium for 2 days, cloning efficiency (the number of cells forming colonies relative to the number of cells plated) was reduced approximately 20 to 30%. When 50 or 100µg caffeine per ml medium were added 1 hr after the carcinogen and allowed to remain for 48 hrs, the absolute number of transformations increased and an enhancement ratio of 3 to 6 resulted (the number of transformations obtained by the double treatment divided by that obtained by carcinogen only). Caffeine added 1 hr prior to the carcinogen did not increase toxicity or alter the transformation frequency. When 0.5 to 200µg caffeine per ml medium was added 1 hr post-AcAAF, cloning efficiency decreased and average number of transformations increased with caffeine concentration. The addition of a constant amount of caffeine (50µg/ml) for 48 hrs at different intervals after carcinogen resulted in maximum enhancement (10 to 17) when caffeine was added at 4 hrs. With AcAAF, the addition of caffeine at later intervals such as 24 and 48 hrs caused no enhancement, whereas with N-methyl-N'-nitro-N-nitrosoguanidine the enhancement was approximately 6-fold. The critical difference between addition of caffeine at 1 and 4 hrs post-carcinogen for 48 hrs is not due to the point at which treatment ended. The addition of caffeine 4 hrs post-AcAAF for different time intervals (4 to 48 hrs) resulted in significant enhancement when the caffeine was added for a minimum of 12 hrs post carcinogen.

Results attempting to associate viral activation with chemical transformation of Syrian hamster cells remain equivocal. Hybridization studies with viral

in vitro complementary DNA (cDNA) can be summarized as follows. cDNA prepared from Kirsten sarcoma-leukemia virus (KiSV) hybridizes 95% to its own RNA and about 85% to RNA from mouse cells infected with and transformed by but not producing KiSV. The same cDNA hybridizes to a maximum of 10% to RNA from hamster embryo cells and hamster chemically transformed cells. Use of this probe was discontinued. cDNA from Harvey virus grown in hamster cells was then used for similar studies in the hopes that the Harvey sarcoma component would acquire an endogenous hamster virus as helper. cDNA from this virus hybridized about 30% to hamster embryo cell RNA, chemically transformed cell RNA, and Harvey virus producer cell RNA. No cDNA discriminates among cell RNA preparations. Because of the difficulty preparing enough cDNA from hamster virus to adequately characterize, it is not possible to say what percent of hamster virus genome is represented in the cDNA. Other approaches have therefore been attempted. Labeled cDNA was prepared from cell RNA using oligo (dT) as a primer for synthesis and AMV reverse transcriptase. cDNA was prepared from chemically transformed cell RNA, from hamster embryos, and from hamster embryo organs. All cDNA's hybridized to a high level to all RNA's. Because of the genetic complexity of the RNA preparations in question, these results are of a preliminary nature, and some sort of recycling experiment must be done.

Investigations aimed at obtaining transformation of human cells with chemical carcinogens have been continued. As reported last year, toxicity, morphologic alterations in cell morphology and extension of lifespan of carcinogen treated fetal cells have been observed. In addition, approximately one cell per 10^6 cells from carcinogen treated cultures will form a progressively growing colony in soft agar. This correlates highly with neoplastic transformation in other models. Agar derived cells have been injected into thymusless nude mice to evaluate the tumorigenic potential of the cells. Thymusless nude mice deficient in xenograft rejection were selected as hosts for evaluation of human cell tumorigenicity after it had been determined in this laboratory that equivalent numbers of chemical carcinogen transformed hamster or guinea pig cells produced progressively growing tumors with nearly the same incidence in the host species and the nude mouse. Noncarcinogenic chemical treated guinea pig and hamster cells that were nontumorigenic in the host species also were nontumorigenic in the nude mouse. To date, however, no human fetal cells derived from colonies in soft agar have produced a tumor in a nude mouse (6-12 months observation). Ordinarily, 90% of the hamster or guinea pig lines produce tumors in this period. The human cells, however, unlike hamster and guinea pig cells from agar derived colonies do not have a definite life span. Several strains that have been serially subcultured ceased cell multiplication 10-20 subcultures after isolation from agar. Transformation as defined for nonhuman mammalian cells is, therefore, incomplete in these human cells despite their ability to grow as colonies in agar, and may explain the failure of the cells to grow as a tumor in the nude mouse.

Due to the moratorium imposed during 1974 on the experimental use of human fetal cells, no further investigations on human fetal cells were performed. In place of the fetal cells, fibroblast-like cell cultures derived from biopsies obtained from pediatric and adult individuals with the disorder xeroderma

pigmentosum (XP) were treated with carcinogen. These cells were selected in view of the abnormal DNA repair processes associated with XP in anticipation that defective DNA repair might facilitate a neoplastic change following carcinogen treatment. Xeroderma cells have been also treated with a combination of physical and chemical carcinogenic insults: 250R X-irradiation followed by carcinogens causing either excision or strand type DNA defects. Morphologic alterations have been observed in the treated cultures which are being subcultured prior to evaluation for formation of cell colonies in agar, lifespan in culture and neoplastic potential.

Significance to Biomedical Research and the Program of the Institute:

The need to study chemical carcinogenesis in vitro is obvious when one realizes that carcinogenesis is really a problem in cell ecology. Consequently, in vitro carcinogenesis provides an opportunity to study chemical-target cell interaction and chemical-nontarget cell problems such as other effects of the chemical on cells without the complications of in vivo problems. Therefore, in vitro carcinogenesis has wide applications in terms of studying compounds in man's environment for the sake of control as well as for determining how they alter the physiological process of cells.

Proposed Course of Project:

It is planned to further study the conditions which are responsible for or can alter in vitro transformation with specific emphasis on the requirements of human fetal cells. These will include the use of X-rays, combinations of carcinogens of different classes, the combination of in vivo - in vitro systems, and the effect of stimulators and inhibitors of mixed function oxidases on toxicity and transformation. The mechanisms of early events of carcinogen-cell interaction will be studied. Investigation of different proximate carcinogens and metabolic inhibitors is planned. In addition, experiments are planned to test whether neoplastic transformation has occurred as a result of direct induction by chemicals, as the result of selection or of viral activation. The testing of the tumorigenicity of cells exposed for different time intervals will continue.

Honors and Awards

Invited Lecture, "Model systems in carcinogenesis." Columbia University, College of Physicians and Surgeons, New York, 1974.

Invited Lecture, "Host-mediated assay." International Symposium on Approaches to Early Detection of Chemical Toxicity, Guildford, England, 1974.

Invited Lecture, "Studies on the quantitation of in vitro transformation of cells." Gene Expression and Carcinogenesis in Cultured Liver, Los Angeles, California, 1974.

Organizer of the Formal Symposium: Carcinogenesis In Vitro for the Twenty-Fifth Annual Meeting of the Tissue Culture Association, Miami Beach, Florida, 1974.

Invited Lecture, "Transformation of mammalian cells in culture by chemical carcinogens." Conference on Chemical Carcinogenesis, XIth International Cancer Congress, Florence, Italy, 1974.

Chairman and Organizer, Symposium 37, In Vitro Carcinogenesis, XIth International Cancer Congress, Florence, Italy, 1974.

Invited Lecture, "In vitro transformation of mammalian cells: Interaction of diverse agents." Carcinogenesis Program, Third Annual Collaborative Conference, Orlando, Florida, 1975.

Publications

Casto, B. C. and DiPaolo, J. A.: Combined chemical and DNA viral carcinogenesis. In Proceedings, XIth International Cancer Congress, Excerpta Medica, Amsterdam, (In Press).

Casto, B. C. and DiPaolo, J. A.: In vitro transformation: Interaction of chemicals, viruses and irradiation. In Ito, Y. and Dutcher, R. M. (Eds.) Comparative Leukemia Research 1973, Tokyo, Karger, Basel, Univ. of Tokyo Press, 1974, pp. 191-193.

DiPaolo, J. A.: Karyological instability of neoplastic somatic cells. In Vitro, (In Press).

DiPaolo, J. A.: Short-term tests for carcinogenesis: Tests involving induction of neoplasia. In Golberg, L. (Ed.): Carcinogenesis Testing of Chemicals. Cleveland, CRC Press, Inc., 1974, pp. 91-94.

DiPaolo, J. A.: Transformation of mammalian cells in culture by chemical carcinogens. In Proceedings, XIth International Cancer Congress, Excerpta Medica, Amsterdam, (In Press).

DiPaolo, J. A.: Mammalian cell models for chemical carcinogenesis. In Montesano, R., and Tomatis, L. (Eds.): Chemical Carcinogenesis Essays. Lyon, International Agency for Res. On Cancer, 1974, pp. 111-118.

DiPaolo, J. A.: In vitro transformation: Interactions of chemical carcinogens and radiation. J. Cell. (Comparative) Physiol., (In Press).

DiPaolo, J. A.: Susceptibility of mammalian cells in vitro to Neoplastic transformation by chemical carcinogens. In Gerschenson, L. E., and Thompson, E. B. (Eds.): Gene Expression and Carcinogenesis in Cultured Liver, New York, Academic Press, (In Press).

DiPaolo, J. A., Donovan, P. J., and Casto, B. C.: Enhancement by alkylating agents of chemical carcinogen transformation of hamster cells in culture. Chem. Biol. Interact. 9: 351-364, 1974.

DiPaolo, J. A., and Popescu, N. C.: Chromosome bands induced in human and Syrian hamster cells by chemical carcinogens. Br. J. Cancer 30: 103-108, 1974.

Donovan, P. J. and DiPaolo, J. A.: Caffeine enhancement of chemical carcinogen-induced transformation of cultured Syrian hamster cells. Cancer Res., 34: 2720-2727, 1974.

Olinici, C. D. and DiPaolo, J. A.: Chromosome banding of rat fibrosarcomas induced by in vitro transformation of embryo cells or in vivo injection of rats by 7,12-dimehylbenz(a)anthracene. J. Natl. Cancer Inst., 52: 1627-1634, 1974.

Olinici, C. D., Evans, C. H., and DiPaolo, J. A.: Chromosome patterns of non-transformed variants from chemically transformed Balb/3T3 cells. J. Cell. Physiol. 83: 401-407, 1974.

Oshiro, Y. and DiPaolo, J. A.: Changes in the uptake of 2-deoxy-D-glucose in Balb/3T3 cells chemically transformed in culture. J. Cell. Physiol. 83: 193-201, 1974.

Popescu, N. C. and DiPaolo, J. A.: G and C chromosomal banding produced sequentially in living and fixed human cells. Lancet 1: 209-210, 1974.

Popescu, N. C., Olinici, C. D., Casto, B. C. and DiPaolo, J. A.: Ramdom chromosome changes following SA7 transformation of Syrian hamster cells. Int. J. Cancer 14: 461-472, 1974

Ts'o, P. O. P. and DiPaolo, J. A., (Eds.): Chemical Carcinogenesis, New York, Marcel Dekker, Inc., 1974, Pt A, 464 pp. and Pt B, 344 pp.

Yamamoto, N., Anderson, M. D. and DiPaolo, J. A.: Phage and bacterial inactivation and prophage induction by chemical carcinogens. Mol. Pharmacol. 10: 640-647, 1974.

Project No. Z01-CP-04673-04-BGY
1. Biology Branch, Carcinogenesis DCCP
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Neoplastic Transformation of Guinea Pig Cells by Chemical Carcinogens In Vitro

Previous Serial Number: Same

Principal Investigator: Charles H. Evans, M.D., Ph.D.

Other Investigators: Joseph A. DiPaolo, Ph.D.

Cooperating Units: None

Man Years:

Total	2.1
Professional	0.6
Other	1.5

Project Description

Objectives:

The primary objective of this project is to establish conditions and methods for the quantitative study of in vitro chemical transformation of guinea pig cells. The investigation complements the previously developed and current in vitro chemical carcinogenesis system established within the Cytogenetics and Cytology Section employing Syrian hamster embryo cells and the tumor immunology program utilizing in vivo chemical carcinogen induced tumors developed in strain-2 guinea pigs.

The specific objectives of the project are twofold:

1. The development of a rapid assay system for chemical carcinogens suitable for the screening of compounds and populations. As an inbred strain with a low incidence of spontaneous malignancy strain-2 guinea pigs offer a mammalian system in addition to the Syrian hamster for the analysis of chemicals as potential carcinogens and investigation of the mechanisms of chemical carcinogenesis at the cellular level.

2. The development of assays to determine whether in vitro chemical transformation is accompanied by the appearance of tumor specific antigens and/or alterations associated with the neoplastic state. The Biology Branch has extensive experience with tumor immunology in strain-2 guinea pigs. A variety

of in vivo and in vitro immunological techniques are presently being utilized. The availability of these techniques and the syngenetic system offered by the inbred strain-2 guinea pig make this system a better choice for immunological study at this time than the Syrian hamster. The development of neoplastic cells transformed in vitro by chemical carcinogens will permit assessment of cell surface antigen alterations associated with chemical carcinogens as well as further understanding of in vivo tumor immunity in strain-2 guinea pigs as a model system for carcinogenesis and tumor immunology in general.

Methods Employed:

The quantitative in vitro chemical carcinogenesis system developed employing Syrian hamster embryo cells within the Cytogenetics and Cytology Section has been utilized throughout this study. Cells are freshly isolated from whole or specific organs of strain-2 guinea pig embryos or fetuses and are grown in the presence or absence of irradiated rat, hamster or strain-2 guinea pig feeder cell layers. The cells as a mass culture or as individual cells are exposed to chemical carcinogen subsequent to seeding the cells in the Petri dish.

Approximately one week subsequent to treatment, colonies are examined under phase contrast microscopy or light microscopy following fixation and staining of the cells for the number of transformed colonies, toxicity and spectrum of morphology of both the normal and transformed colonies.

Major Findings:

1. The development of toxicity following the addition of chemical carcinogens in both mass and cloned cell cultures was similar to that observed in hamster cells exposed to the same chemicals. Carcinogens from several chemical classes have been employed including the polycyclic compounds benzo(a)pyrene, 3-methylcholanthrene and 7,12-dimethylbenz(a)anthracene as well as the nonpolycyclic chemicals such as N-acetoxy-N-2-fluorenylacetamide and N-methyl-N-nitro-N-nitrosoguanidine. Fetal cells have also been exposed to chemical carcinogen while in utero via the transplacental route following intraperitoneal inoculation of pregnant guinea pigs. The procedure is the same as that developed for the transplacental host-mediated in vivo - in vitro hamster bioassay with excision of the guinea pig fetuses 48 hrs subsequent to maternal inoculation and identification of transformation following introduction of the fetal cells into culture.

2. Morphological cellular changes of guinea pig cells exposed to chemical carcinogens and compatible with in vitro chemical neoplastic transformation as observed in Syrian hamster embryo cells have been seen with cells exposed to chemical carcinogen but not to noncarcinogens either while in utero or after introduction into culture. Twenty-four cell strains have been evaluated during 12-24 months of cultivation. Morphological alterations in carcinogen treated cultures appear shortly after treatment, but transformation, a loss of cell orientation does not appear for four or more months, is not seen in controls, and may precede by 2-6 months development of the capacity of transformed cells to grow as tumors in irradiated newborn guinea pigs. Chromosome alterations,

changes in plating efficiency, saturation density and doubling time of transformed cells in medium containing 10% serum are not uniformly associated with progressive tumor growth. The ability of transformed cells to form colonies in 0.35% agar appears to be the best index of tumorigenic potential. Frequently the capacity to grow as colonies in agar and produce tumors does not occur until 6-12 months following exposure to carcinogens such as benzo(a)-pyrene, 3-methylcholanthrene or N-methyl-N'-nitro-N-nitrosoguanidine. Controls originally exposed to noncarcinogens including acetone, pyrene or anthracene do not form agar colonies or tumors after inoculation of 10^8 cells. Experience to date with guinea pig cells indicates that the changes in some growth parameters occur prior to expression of tumor growth. Formation of colonies in agar may more closely relate to tumorigenic potential than other commonly assessed properties of cells in culture.

3. Transformation of guinea pig cells, as in other model systems employing hamster, mouse or rat cells, by chemical carcinogens appears inductive rather than by selection of pre-existing neoplastic cells. Noncarcinogen treated cells had normal morphology and did not form colonies in agar nor tumors in vivo during 20 months of continuous subculture. Whether the carcinogen is solely responsible or acts in concert with a latent virus is an unresolved question. Intracellular and extracellular virus-like particles have been observed in all guinea pig cultures examined after BRdU treatment, including nontransformed, nontumorigenic solvent treated, transformed nontumorigenic carcinogen treated and transformed tumorigenic carcinogen treated. The particles exhibit both similarities to and differences from the A, B and C particles of other species. No apparent association has been found between the presence of the particles in guinea pig cell cultures and tumorigenic potential of the cells.

4. Several new short-term assays that correlate with the tumorigenic potential of transformed cells have been developed. A cytotoxic assay measuring the amount of radioactivity released from tritiated thymidine labeled target cells in the presence of phytohemagglutinin stimulated mineral oil induced peritoneal exudate cells from healthy unimmunized syngeneic guinea pigs shows that each tumorigenic cell line and its tumor-derived cell line share a similar pattern of release; tumorigenic cell lines as a group, release a greater quantity of radioactivity than do nontumorigenic transformed or nontransformed cell strains. Colony formation by transformed tumorigenic cells is also inhibited by mineral oil induced peritoneal exudate cells from unimmunized syngenic guinea pigs in a colony inhibition assay. Colony formation by nontumorigenic cells, including early passage untreated fetal cells, cells originally treated with noncarcinogenic chemicals and nontumorigenic chemical carcinogen induced morphologically transformed cells, is not inhibited and in some cases is enhanced. The inhibitory factor(s) is soluble and stable during storage at -35°. This permits analysis of multiple lines at different times with the same preparation of peritoneal cell soluble colony inhibitory factor(s). In a third assay it has been observed that the skin reaction to tumorigenic cells is greater than that obtained with nontumorigenic cells during the four days following intradermal inoculation of the cells into normal unimmunized guinea pigs. The in vitro cytotoxicity and colony inhibition assays mediated by nonimmune peritoneal exudate cells and the in vivo differential skin reactivity in nonimmune

animals are being further explored to determine the value of these methods in comparison with growth of cells as colonies in agar as indices of the tumorigenic potential of cells and as additional means complementing the assessment of antigenic changes in the cells to evaluate cell surface alterations during the transition to neoplasia.

Significance to Biomedical Research and the Program of the Institute:

This project extends the current system of in vitro chemical carcinogenesis within the Cytogenetics and Cytology Section to an additional mammalian system. It also broadens the in vivo chemical carcinogenesis investigations within the Cellular Immunity Section to include in vitro chemical neoplastic transformation of strain-2 guinea pig cells. Development of this system will permit further opportunity to study chemical target cell interaction without the complications inherent in in vivo analysis. In vitro chemical neoplastic transformation of strain-2 guinea pig cells will provide another assay system in addition to the Syrian hamster for screening of chemicals as potential carcinogens and broaden our understanding of chemical carcinogenesis at the cellular level through inspection of intracellular and cell surface alterations accompanying neoplastic transformation. Development of this new model offers an additional avenue for studying the process of carcinogenesis using a species with established tumor biology in which spontaneous transformation has not been seen, which possesses well defined immunological parameters, and in which discrete stages in the transition from the growth controlled to the neoplastic state may be studied.

Proposed Course of Project:

Conditions and methods will continue to be refined toward attaining optimal development of in vitro transformation of strain-2 guinea pig cells as a reproducible, quantitative and reliable assay for potential chemical carcinogens. Investigations will continue in defining the events, factors and cellular alterations accompanying the stages, i.e. morphologic alteration, morphologic transformation and potential for neoplastic growth, in the transition from the growth controlled to the neoplastic state. Of immediate interest are the properties of cells with neoplastic potential that permit them to grow as colonies in agar and to be susceptible to the cytotoxic and growth inhibitory activities mediated by nonimmune leukocytes in the absence of or independent of the presence of detectable tumor specific cell surface antigens. The elucidation of these relationships will provide further understanding of the mechanism of chemical carcinogenesis at the cellular levels of tumor establishment and immunity in the host animal.

Honors and Awards

Invited speaker, Symposium on In Vitro Carcinogenesis, XIth International Cancer Congress, Florence, Italy, October, 1974.

Publications

Evans, C. H., Cooney, A. M. and DiPaolo, J. A.: Colony inhibition mediated by nonimmune leukocykes in vitro and skin reactivity in vivo as indices of tumorigenicity of guinea pig cultures transformed by chemical carcinogens. Cancer Res. (In Press).

Evans, C. H. and DiPaolo, J. A.: Neoplastic transformation of guinea pig fetal cells in culture induced by chemical carcinogens. Cancer Res. (In Press).

Evans, C. H. and DiPaolo, J. A.: Properties of cells from different species accompanying chemical carcinogen induced transition to the neoplastic state. In Proceedings, XIth International Cancer Congress, (In Press).

Hsiung, G. D., Fong, C. K. Y. and Evans, C. H.: Prevalence of endogenous oncornavirus in guinea pigs. Intervirology 3: 319-331, 1975.

Zwilling, B. S., Meltzer, M. S. and Evans, C. H.: Differential cytotoxicity of tumorigenic and nontumorigenic strain-2 guinea pig cells mediated by syngeneic phytoheumgglutin stimulated peritoneal exudate cells. J. Natl. Cancer Inst. (In Press).

Project No. Z01-CP-04576-01-BGY
1. Biology Branch, Carcinogenesis, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Modification of Tumor Cells

Previous Serial Number: None

Principal Investigator: Sarkis H. Ohanian, Ph.D.

Other Investigators: Tibor Borsos, Sc.D., Michael D. P. Boyle, Ph.D., Berton Zbar, M.D., Robert C. Bast, Jr., M.D., Mtutuzeli Xuma, M.D. and Martin Segerling, M.D.

Cooperating Units: Institute of Hygiene, Hamburg, Germany

Man Years:

Total	:	2.00
Professional	:	1.75
Other	:	0.25

Project Description

Objectives:

1. To study the mechanisms whereby enzymes and metabolic inhibitors increase the sensitivity of tumor cells to immune attack.

2. To determine the physical and chemical processes and cell products which may be modified following such treatment.

3. To determine the malignant properties of tumor cells treated in vitro and in vivo with metabolic inhibitors or enzymes.

Methods Employed:

Antibody is quantitated by C1FT and immune cytolysis. Sensitivity to attack by cells is measured by incorporation or release of ^{125}IUDR. Incorporation of radiolabelled precursor of DNA, RNA and protein as being used to measure general metabolic properties of the cell.

Tumor growth in vivo is used to measure the malignant properties of tumor cells.

Major Findings:

Line-10 tumor cells pretreated with metabolic inhibitors or enzymes of selected specificity are rendered susceptible to killing by anti-Forssman, rabbit antitumor, or guinea pig antitumor antibody and GP complement. The effect is dose and temperature dependent and is reversible. The increase in sensitivity is not due to an increase in antigen expression as measured by the C1FT test. The enzymes which are most effective are proteolytic, such as the papain family. Lipase, hyaluronidase and collagenase have yielded varied results.

Methods for measuring antibody dependent cell mediated killing have been developed. The assay is based on the release of ^{125}IUDR from prelabelled target cells. Approximately 30% release of label is noted with tumor cells not treated with enzymes or metabolic inhibitors. The attacking cells are peritoneal exudate cells of normal guinea pigs. The adherent cell population is the active cell while the nonadherent population appears to prevent the adherent cells from killing the tumor cells. No antitumor cell activity was seen with spleen cells from normal guinea pigs and no enhanced killing of tumor cells was observed by peritoneal exudate cells or by spleens from immune animals.

Tumor cell growth in the skin of guinea pigs can be inhibited by injection of selected metabolic inhibitors into the developing tumor. Injection of inhibitors intraperitoneally into guinea pigs with skin tumors does not affect tumor growth. Tumor cells treated in vitro with actinomycin D or adriamycin will grow in the skin of guinea pigs then regress while control (untreated) cells continue to grow and kill the animal. Preliminary results suggest that coating of treated tumor cells with antitumor antibody is of limited value in preventing the growth of tumor cells.

Significance to Biomedical Research and the Program of the Institute:

Modification of tumor cells by metabolic inhibitors, enzymes and chemotherapeutic agents furnished a new tool to study the interaction of tumor cells with the immune defense mechanisms of the host.

Proposed Course of Project:

Radioisotope incorporation studies will be continued as probes to determine the cellular functions which are modified by treatment with enzymes or inhibitors.

Enzymes of differing specificity will be examined for their effect on the sensitivity of cells to antibody-complement mediated killing.

The structure and chemical atributes of normal cells, tumor cells and treated tumor cells will be studied by immunohistochemistry and immunoelectronmicroscopy.

Work will continue on the effect of enzymes and of inhibitors of macromolecular synthesis on the growth of tumor cells in vivo and in vitro.

Project No. Z01-CP-4639-09-BGY
1. Biology Branch, Carcinogenesis, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mechanism of Complement Fixation and Action

Previous Serial Number: Same

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: Herbert J. Rapp, Sc.D., Mtutuzeli Xuma, M.D., Alain M. Bernard, M.D., and Sarkis H. Ohanian, Ph.D.

Cooperating Units: Direction Generale de la Recherche Scientifique et Technique, Paris, France

Man Years:

Total	2.0
Professional	1.5
Other	0.5

Project Description

Objectives:

To apply rational complement fixation tests based on the fixation and transfer of the first component of complement to the analysis of antigen-antibody reactions, in particular in the search for cancer specific antigens.

Methods Employed:

The model for studying cytotoxic reactions mediated by antibody and complement consists of sheep erythrocytes, hemolytic antibody and guinea pig complement. Purification procedures for antibodies and the complement components include: preparative (large scale) gel filtration, ion exchange chromatography and preparative free electrophoresis. Other techniques used include precipitin and immunoelectrophoretic analysis, analytical, zonal and preparative ultracentrifugation and other immuno- and physico-chemical methods.

Major Findings:

The ultimate goal of this work is the development of immunological tools that permit exploration of (1) immunological approaches to cancer diagnosis and (2) possible host immune defense mechanisms against cancer.

The finding reported in previous years that normal rabbit serum contains naturally occurring anti-guinea pig tumor-specific complement-fixing antibody was confirmed. Although the antibody is complement-fixing, it is present in such low amounts that it cannot be detected by cytotoxic activity. Sera of several rabbits were screened for the presence of the natural antitumor antibody. It was found that the amounts of antibody vary widely from rabbit to rabbit ranging from nondetectable to relatively high levels of activity. Fractionation of the serum on Sephadex G-200 showed that the antibody activity was in the excluded portion of the serum. Studies are also conducted into the origin of the natural antitumor antibody in the rabbits.

Recently a new factor inhibiting the action of C1, the first component of complement, was identified. It is a product of normal lymph node and spleen cells. The factor is capable of binding to sheep red cells; the site of binding is not known at present. The bound factor is capable of binding precursor and activated C1. Precursor C1 bound by the factor is not recoverable in an active form; bound activated C1 can be removed in active form. The factor thus behaves with respect to activated C1 like a bound complement-fixing antibody but also like an inhibitor for precursor C1. The physical-chemical nature of the new factor is under investigation.

Significance to Biomedical Research and the Program of the Institute:

Complement fixation is one of the most widely used diagnostic tools. The development and successful application of a radically new and very sensitive complement fixation test, the C1FT test, opened up new possibilities in determining antibody-antigen reactions on cell surfaces.

Furthermore, cytotoxic reactions due to antibody and complement are prime examples of body defense mechanisms. Fundamental research into the nature and mechanism of complement fixation and action will contribute greatly to the development of diagnostic tools and to the understanding of the mechanism of immune body defenses.

Proposed Course of Project:

This is a long-range project, and little change is expected in the scope of the work during the next few years. The ultimate goals of these projects are the development of better diagnostic tools based on fixation of complement and the elucidation of molecular events associated with the action of complement and antibodies. It is hoped that as a result of our program of inquiry into the basic problem of the interaction of antibodies, antigens and components of complement, tools will be developed that are of practical significance in the search for cancer antigens.

Publications

Borsos, T. and Ohanian, S. H.: Brief communication: Guinea pig tumor-specific natural antibodies in normal rabbit sera. J. Natl. Cancer Inst. 53 1827-1829, 1974.

Colten, H. R. and Borsos, T.: Biosynthesis of the second and fourth components of complement: Inhibition in vitro by chemical carcinogens. J. Immunol. 112: 1107-1114, 1974.

Detrick-Hooks, B., Borsos, T. and Rapp, H. J.: Quantitative comparison of techniques used to measure complement-mediated cytotoxicity of nucleated cells. J. Immunol. 114: 287-290, 1975.

Project No. Z01-CP-04662-06-BGY
1. Biology Branch, Carcinogenesis, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immune Cytolysis

Previous Serial Number: Same

Principal Investigator: Sarkis H. Ohanian, Ph.D.

Other Investigators: Tibor Borsos, Sc.D., Martin Segerling, M.D., Michael D. P. Boyle, Ph.D., and Charles H. Evans, M.D., Ph.D.

Cooperating Units: Institute of Hygiene, Hamburg, Germany

Man Years:

Total	:	1.35
Professional	:	1.10
Other	:	0.25

Project Description

Objectives:

A study of the molecular aspects of antibody-complement lysis and antigen topography of nucleated cells.

Methods Employed:

Antibodies to normal and tumor antigens are detected and quantitated by immune cytolysis, colony inhibition, C1 fixation and transfer (C1FT) and by uptake of I^{125} labelled antibody.

Immunochemical methods including Sephadex and DEAE chromatography, electrophoresis, immunodiffusion and ultracentrifugation are employed to isolate antibody protein and complement components.

Major Findings:

The malignant guinea pig cells, line-1, line-10 and L_2C appear to express less Forssman antigen than their normal tissue counterparts. Both line-1 and line-10 appear to have the same amount of Forssman antigen. Line-10 sensitized with anti-Forssman antibody is resistant to lysis by complement (C) while

line-1 and L_2C are not. By C1FT tests both line-1 and line-10 sensitized with anti-Forssman antibody have the same number of C1 fixing sites per cell. Line-10 cells are lysable by C, however, when sensitized with specific anti-line-10 antibody.

Line-10 cells sensitized with anti-Forssman or specific rabbit anti-line-10 antibody are killed by human C (HuC) but not guinea pig C (GPC). At dilutions of antibodies generating approximately 10^5 C1 fixing sites per cell, the number of GP and Hu C4 and C3 molecules per cell were determined. Large numbers of these C components (approximately 10^6 C4 and 3×10^5 to 10^6 GPC3 and HuC respectively) were bound to the cells.

Line-1 cells sensitized with anti-Forssman antibody are more sensitive to lysis by HuC and GPC than line-10. However, as with line-10 cells, HuC gives a higher degree of lysis than GPC. No correlation was found between the number of complement fixing sites, the number of C4 and C3 bound and the lysability of the cells.

Treatment of line-1 and line-10 tumor cells with metabolic inhibitors or drugs commonly used in the treatment of cancer has been found to render the cells sensitive to killing by antibody (xenogeneic or syngeneic) and GPC. Some inhibitors were found to increase the sensitivity to killing by anti-Forssman antibody and GPC, but not specific antitumor antibody and GPC. The effect of inhibitors was dependent on concentration of inhibitors, temperature, time and was reversible. Preliminary results indicate the increased sensitivity was not due to increased expression of antigen, fixation of the GPC components C4 and C3 or changes in fluidity of the membrane. This latter property was measured by the ability of tumor cell surface antigens to aggregate when interacted with antibody.

Tumor cells preincubated with selected proteolytic enzymes are rendered susceptible to killing by xenogeneic and syngeneic antibody and GPC. Thus far trypsin, DNAse or RNAse treated cells are not rendered susceptible. This effect is dependent on concentration of enzyme, temperature, time and is reversible. Preliminary results suggest the increased killing is not due to an apparent increase in surface antigen expression.

Several clones of line-1 and line-10 cells in culture were compared with fresh ascites cells in terms of antigen expression and sensitivity to killing by antibody and C. Antigen expression as measured by C1FT or fluorescent microscopy showed the ascites cells to be a heterogenous population of cells while the cloned lines were less heterogenous. Forssman and tumor-specific antigens were found to persist in culture. Cloned lines had the same or less than the ascites forms.

Significance to Biomedical Research and the Program of the Institute:

The elucidation at the molecular level, of the effects of antibody and complement on both normal and malignant cells may be important in understanding control of cell growth by immunologic mechanisms.

Control of tumor cell growth in vitro makes it possible to study the action of specific and nonspecific agents on the cells at different stages of their growth cycle. The knowledge obtained from in vitro experiments will be very helpful when applied to the study of more complicated in vivo conditions.

Proposed Course of Project:

More extensive work will be carried out on the effect of antibody and complement on lysis of nucleated cells. This will include class of antibody (both Forssman and antitumor) and binding of complement components.

Inhibitor and enzyme treatment of line-1 and line-10 cells will be carried out and the effect on macromolecular synthesis in more detail. The surface topography of tumor cells rendered susceptible to killing by antibody and GPC will be studied by immunoelectronmicroscopy.

Publications

Evans, C. H., Ohanian, S. H. and Cooney, A. M.: Tumor-specific and Forssman antigens of guinea pig hepatoma cells: Comparison of tumor cells grown in vivo and in vitro. Int. J. Cancer 15: 512-521, 1975.

Ohanian, S. H. and Borsos, T.: Lysis of tumor cells by antibody and complement. II. Lack of correlation between amount of C4 and C3 fixed and cell lysis. J. Immunol., (In Press).

Segerling, M., Ohanian, S. H. and Borsos, T.: Brief communication: Effect of metabolic inhibitors on killing of tumor cells by antibody and complement. J. Natl. Cancer Inst. 53: 1411-1413, 1974.

Segerling, M., Ohanian, S. H. and Borsos, T.: Chemotherapeutic drugs increase killing of tumor cells by antibody and complement. Science 188: 55-57, 1975.

Project No. Z01-CP-04674-03-BGY
1. Biology Branch, Carcinogenesis, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Antigens of Guinea Pig Cells Transformed In Vitro by Chemical Carcinogens

Previous Serial Number: Same

Principal Investigator: Sarkis H. Ohanian, Ph.D.

Other Investigators: Charles H. Evans, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total	0.2
Professional	0.2
Other	0.0

Project Description

Objectives:

To determine whether surface antigen expression of guinea pig cells transformed in vitro by chemical carcinogens differs from that of untransformed cells.

Methods Employed:

Antibodies to normal and chemically transformed cells are produced in guinea pigs and rabbits. Specificity of the antisera is determined by absorption and tested by complement lysis of cells, immunofluorescence and by quantitative immunochemical techniques.

Major Findings:

Cloned lines derived from five cultures of strain-2 guinea pig cells transformed in culture by benzo(a)pyrene, 3-methylcholanthrene, N-methyl-N-nitro-N-nitrosoguanidine or diethylnitrosamine were studied for the presence of neoantigens (tumor associated and/or specific). Methods of analysis including preparation of reagents previously demonstrated to be suitable for detecting tumor-specific antigen on line-1 and line-10 cells were used.

Rabbit antisera to transformed cells collected two weeks after the immunization schedule were heated to 56°C and absorbed with sheep red blood cells and fresh adult strain-2 guinea pig liver-spleen mixture. Samples were additionally absorbed with autologous or heterologous transformed guinea pig cells. These antisera were assayed for antibody activity by indirect fluorescence microscopy and the quantitative C1FT test and found to have no detectable antibody specific for the individual transformed lines.

Skin tests in strain-2 guinea pigs immunized with X-irradiated or viable cells were also performed. The response to inoculation of test doses of autologous and heterologous cells was not significantly greater than that found in the unimmunized animals.

The transformed cell lines were found to have Forssman and other antigens shared by normal guinea pig cells. The immunofluorescence, C1FT and delayed hypersensitivity skin tests indicate no detectable neoantigens on any cell line.

Significance to Biomedical Research and the Program of the Institute:

These studies may help to determine whether or not antigenic changes of cells associated with neoplastic transformation require host reactions. The ability to detect antigenic changes on cells transformed in vitro would make it possible to relate the time the changes occur to the steps in carcinogenesis. If changes occur relatively early, they might serve as a criterion for defining a precancerous state.

Proposed Course of Project:

In attempts to produce antisera of higher titer, additional immunization procedures will be employed. Several new cell lines transformed with different classes of carcinogens will be studied.

Project No. ZO1-CP-04676-03-BGY
1. Biology Branch, Carcinogenesis, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Detection of Complement Components on Nucleated Cell Surfaces

Previous Serial Number: Same

Principal Investigator: Sarkis H. Ohanian, Ph.D.

Other Investigators: Tibor Borsos, Sc.D. and Berton Zbar, M.D.

Cooperating Units: None

Man Years:

Total	: 0.5
Professional	: 0.5
Other	: 0.0

Project Description

Objectives:

1. To determine whether tumor cells of human (Hu) and guinea pig (GP) origin have detectable complement (C) components on their cell surfaces.

2. To develop techniques for the detection of other complement components bound to the cell surfaces.

Methods Employed:

A new method has been developed to detect human and guinea pig C4 and C3 bound to cell surfaces. Immunochemical methods including DEAE chromatography, electrophoresis and immunodiffusion are employed to isolate antibodies, protein and complement components. Quantitation of antibody and complement components are carried out using radioisotopic labelling quantitative precipitin procedures, C1FT tests, and new immunochemical tests designed to measure C4 and C3.

Major Findings:

Hu and GP C4 and C3 can be bound to the surface of sheep erythrocytes and remain bound for up to two weeks. A test based on inhibition of lysis of these cells is capable of detecting as little as 6×10^{11} Hu and GP C4 molecules and 10^{11} Hu and GP C3 molecules.

Two diethylnitrosamine induced guinea pig hepatoma cells, line-1 and line-10, harvested from the peritoneal cavity of guinea pigs, have detectable GP C4 and C3 bound to their surfaces. No Hu C4 or C3 could be detected on either cell line. Line-10 cells harvested on different days after implantation were examined for bound C4, C3 and host immunoglobulin. Increasing amounts of both C4 and C3 were detected on cells collected on days 3, 6, 10 and 13 after implantation. On day 15 after intraperitoneal injection there was a decrease. The immunoglobulin level, as determined by indirect C1FT tests remained the same up to day 13 when two times as much was detected. On day 15 the immunoglobulin levels decreased slightly from that observed on day 13. Preliminary evidence indicates the class of immunoglobulin may be of the IgG class. Line-10 cells collected 10 days after implantation and incubated at 4°C and 37°C in culture medium were assayed 1 and 2 days later for persistence of the guinea pig complement components and immunoglobulin. Both C4 and C3 as well as immunoglobulin were lost from the surface of the tumor cells. Loss of cell-bound components occurred at 4°C and 37°C, with the greatest loss occurring with cells incubated at 37°C. Cell viability dropped about 30% at 37°C and about 10% at 4°C on the second day.

Line-10 tumor cells sensitized with rabbit anti-Forssman antibody were assayed for the persistence of the immunoglobulin after incubation at 37°C and 4°C. Loss of C1 fixing activity of cell bound antibody could be detected within 6 hours after incubation of cells at 37°C. By the indirect C1FT method no decrease in the amount of immunoglobulin bound to these cells was detectable until after 1 day of incubation. Lesser amounts of immunoglobulin were lost on cells held at 4°C for up to two days. Treatment of tumor cells with metabolic inhibitors had no measurable effect on cell bound C components.

Significance to Biomedical Research and the Program of the Institute:

Techniques for detecting bound complement components may be useful in determining whether a humoral immune response has been produced to tumor cells in vivo. The ability to detect minute quantities of antibodies to tumor cells may provide means of relating humoral immune responses to antitumor effects in vivo.

Proposed Course of Project:

Normal tissue and primary explants of a variety of human and selected guinea pig tumors will be examined for cell-bound C4. Techniques will be developed to detect complement components other than C3 and C4. Additional studies concerning the fate of cell-bound immunoglobulin and complement components will be carried out.

Publications

Ohanian, S. H. and Borsos, T.: Antigenic relationship between the fourth component of human and guinea pig complement. J. Immunol. 114: 161-164, 1975.

Ohanian, S. H. and Borsos, T.: Immunochemical quantitation of cell bound C4. J. Immunol. 112: 979-986, 1974.

Ohanian, S. H. and Borsos, T.: Immunochemical quantitation of the third component of guinea pig complement in fluid phase and bound to cell surfaces. J. Immunol. 114: 326-330, 1975.

Project No. Z01-CP-04667-05-BGY
1. Biology Branch, Carcinogenesis, DCCP
2. Tumor Antigen Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Isolation and Study of Tumor-Specific Antigens

Previous Serial Number: Same

Principal Investigators: Edward J. Leonard, M.D. and Barbara Detrick-Hooks, Ph.D.

Other Investigators: Tibor Borsos, Sc.D., Monte S. Meltzer, M.D. and Howard G. Smith, M.D.

Cooperating Units: None

Man Years:

Total	: 1.2
Professional	: 1.2
Other	: 0.0

Project Description

Objectives:

To obtain tumor-specific antigens in soluble and partially purified form. To use these antigens in inducing, establishing and measuring tumor-specific immunity in guinea pigs. Knowledge derived from the guinea pig model will be applied to the isolation, purification and characterization of human tumor-specific antigens. These antigens will then be used in diagnostic studies of human tumor epidemiology, in monitoring patient response to treatment and as an aid in treatment. Initial studies will be on the ascites cell form of diethylnitrosamine induced hepatoma of strain-2 guinea pigs.

Methods Employed:

Initial extraction: 3M KCl and other hypertonic salts. Further purification: ammonium sulfate precipitation, gel filtration, DEAE chromatography. Assay methods: skin testing; in vitro correlates of cellular immune responses: lymphocyte blastogenesis, inhibition of macrophage migration; immunization of guinea pigs with extracts and challenge of test animals with live tumor cells. Production and testing of heterologous antisera to tumor antigen: immunization of rabbits with whole tumor cells, absorption of sera with various cell suspensions, testing of absorbed sera by C1 fixation and transfer test and by immune fluorescence.

Major Findings:

Heterogeneity of line-10 antigen extracts: the heterogeneity of 3M KCl extracts of line-10 tumor cells was documented by both Sephadex G-200 and DEAE chromatography. This heterogeneity underlines the need to find a more satisfactory solubilization step before further purification leading to study of TSTA structure.

Antigenicity and immunogenicity of line-10 KCl extracts and ghosts were studied. Antigenicity was measured by capacity of the preparations to induce delayed cutaneous hypersensitivity (DCH) reactions in line-10 immune guinea pigs. Immunogenicity was measured by the capacity of extracts or ghosts to immunize guinea pigs so that they would reject a line-10 tumor challenge. When ghosts or extracts were compared with viable cells, there was a remarkable discrepancy between antigenicity and immunogenicity: 700 times more antigen in the form of KCl extract was required to equal the effect of viable cells in protecting animals against tumor challenge. It is not known whether this difference is due to an alteration in the quality of the antigen in the extract, to continued production of antigen by viable cells to make up the 700-fold difference, to differences in mode of presentation to antigen-reactive cells or to the presence of two antigens, one that produces delayed hypersensitivity and one that induces in vivo tumor immunity.

Line-10 and line-1 tumor antigens were isolated from the ascitic fluid of guinea pigs bearing these tumors and from exhausted tissue culture media of cultured tumor cells. These tumor antigens can be isolated without extraction procedures. After a 10-fold concentration line-10 ascites and tissue culture fluids yield a precipitation in agar gel with specific rabbit anti-line-10 antiserum. Absorption of this serum with normal guinea pig serum, normal guinea pig peritoneal exudate cells, guinea pig fetal cells or line-1 tumor cells does not alter the lines of precipitation formed with concentrated ascitic or tissue culture fluids. Immunodiffusion data indicate that more than one tumor antigen may be present since two lines of precipitation were detected. Ascitic fluid and tissue culture fluids from line-1 cells fail to precipitate in gel diffusion with either line-1 or line-10 antibody. However, antigen activity can be demonstrated for both line-10 and line-1 tumor antigens by tumor line specific inhibition of cytotoxicity and immunofluorescence.

Tumor antigens from line-10 and line-1 have been partially purified by gel filtration through Sephadex G-200 and antigen activity was detected in the excluded portion of the eluate by inhibition of cytotoxicity and immunofluorescence.

Line-10 tumor antigen was subjected to ultracentrifugation at 20,000 x g for 30 minutes and the supernatant and pellet fractions checked for antigen activity. Since antigen activity was detected in the supernatant fluid, this fraction was centrifuged at 300,000 x g for one hour. The antigen was not sedimented after this procedure.

In addition to these three in vitro assays, antigen activity was also studied by delayed hypersensitivity reactions in guinea pigs immune to the appropriate tumors. Preliminary experiments indicate that the tumor antigen preparations induce specific skin reactions in the immune guinea pigs.

Significance to Biomedical Research and the Program of the Institute:

The experimental approach that documented heterogeneity of line-10 antigen extracts provides a model that should be used by other investigators to determine whether they have antigen suitable for further purification and structural studies. The results on yield and heterogeneity point up the need for new and improved solubilization methods. The discrepancy between antigenicity and immunogenicity of KCl extracts or line-10 ghosts compared with viable cells underlines another problem that must be solved if cell-free preparations are to be used for active immunization.

The studies on the "naturally solubilized" antigens permit further characterization and immunochemical analysis of the line-10 and line-1 tumor antigens. Their presence in the tumor-bearing host and its immune response to them could offer one approach for a better understanding of the immune events in host tumor interactions.

Proposed Course of Project:

Further studies on the immunogenicity of non-viable cells and subcellular preparations. Use of other methods for antigen solubilization. Further studies on the molecular nature of the soluble antigen and study of the biological significance of the presence of soluble antigens in the tumor-bearing host.

Publications

Kripke, M. L. and Borsos, T.: Brief communication: Accelerated development of benzo(a)pyrene-induced skin tumors in mice treated with pyran copolymer. J. Natl. Cancer Inst. 53: 1409-1410, 1974.

Kripke, M. L and Borsos, T.: Guest editorial: Immune surveillance revisited. J. Natl. Cancer Inst. 52: 1393-1395, 1974.

Kripke, M. L. and Borsos, T.: Immunosuppression and carcinogenesis. A review. Isr. J. Med. Sci. 10: 888-903, 1974.

Leonard, E. J., Meltzer, M. S., Borsos, T. and Rapp, H. J.: Immunogenicity of soluble tumor-specific antigen. In Immunological Aspects of Neoplasia, Proceedings of the Twenty-Sixth Annual Symposium on Fundamental Cancer Research. Baltimore, Williams & Wilkins Publishing Co., (In Press).

Project No. Z01-CP-04675-03-BGY
1. Biology Branch, Carcinogenesis, DCCP
2. Tumor Antigen Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunological Mechanisms of Tumor Rejection

Previous Serial Number: Same

Principal Investigators: Edward J. Leonard, M.D. and Monte S. Meltzer, M.D.

Other Investigators: Robert W. Tucker, M.D., Glenn E. Trivers, David A. Boetcher, M.D., and Mary Stevenson.

Cooperating Units: Laboratory of Biology, NCI

Man Years:

Total	2.5
Professional	2.5
Other	0.0

Project Description

Objectives:

To determine what immunological mechanisms occur in tumor cell killing in order to provide a rational basis for elimination of tumor cells by immunological means.

Methods Employed:

Chemically-induced murine and guinea pig tumors, maintained in tissue culture or by passage in syngeneic hosts were used. Effector immune responses were measured by 1) cytotoxicity assays based on the release of radioactive label from damaged target cells and 2) macrophage chemotactic responses to complement or lymphocyte-derived chemotactic stimuli.

Major Findings:

1. Specific antigen or mitogen-stimulated guinea pig peritoneal exudate (PE) cells were cytotoxic to syngeneic target cells in vitro. This cytotoxicity reaction is immunologically nonspecific in that the target cells were antigenically unrelated to the stimulus which initiated the immune response. In collaboration with the Cytogenetics and Cytology Section, the susceptibility of several tumorigenic and nontumorigenic syngeneic cell lines to this cytotoxic reaction was examined. Cytotoxicity was estimated

by measuring the release of ^{3}H-thymidine from pre-labeled target cells. Using optimal concentrations of mitogen and PE cells, all tumorigenic cell lines were more affected than the non-tumorigenic cell lines. Non-tumorigenic cells which included: 1) short and long-term cultured embryo-derived cells, 2) chemical carcinogen-exposed and untreated cells and 3) cytologically normal and abnormal (transformed) cells, had less specific release of radioactive label than the tumorigenic cells. Target cell susceptibility to this cytotoxic reaction was directly related to the tumorigenicity of the cell line.

2. Peritoneal macrophages from mice infected with Mycobacterium bovis, strain BCG are cytotoxic to tumor cells in vivo and in vitro. These BCG-activated macrophages affect nonneoplastic cells to a lesser degree. The differential susceptibility of neoplastic and nonneoplastic cells to BCG-activated macrophages was confirmed for syngeneic, allogeneic and xeno-geneic tumor cells derived from chemical or viral-carcinogen or spon-taneously induced neoplastic changes. In collaboration with the Cell Physiology and Oncogenesis Section (Laboratory of Biochemistry, NCI), the interaction of BCG-activated macrophages with neoplastic and non-neoplastic cell lines in vitro was examined in detail. Neoplastic and non-neoplastic cell lines each derived from the same cloned syngeneic embryo cell were used as a model system. Macrophage-target cell interaction was analyzed by phase-contrast cinemicrography and by the release of ^{3}H-thymidine from prelabeled target cells. Analysis of macrophage-target cell cultures revealed the following: 1) BCG-activated macrophage-tumor cell interaction was characterized by repeated and relatively short-lived (about 2 hours) cell to cell contacts inducing complete and permanent tumor cytostasis, degenerative morphological changes and eventual cell lysis. BCG-activated macrophages were partially cytostatic to nonneoplastic cells but degenerative morphological changes or target cell death was not observed. Macrophages from uninfected (control) mice had little effect on either target cell line, 2) the translational movement (an index of cell motility) of BCG-activated macrophages on tumor cell monolayers was up to 5 times the movement observed on nonneoplastic target cells. This increased macro-phage motility among tumor cells was not observed with control macrophages, 3) peritoneal cells from BCG-infected mice were cytotoxic to tumor cells in vitro as measured by release of ^{3}H-thymidine. The cell responsible for tumorcytoxicity was the substrate-adherent macrophage. Macrophages from uninfected mice or from oil, starch or thioglycollate induced PE had little effect on tumor monolayers, 4) tumoricidal macrophages were present at 3-7 days and persisted intraperitoneally through 6 weeks after a single BCG injection, 5) two neoplastic/nonneoplastic cell line pairs (all four lines derived from the same cloned syngeneic embryo cell) were used as target cells. Both tumor cell lines had significantly more release of ^{3}H-thymidine than did either nonneoplastic cell line. Furthermore, in a mixed neoplastic/nonneoplastic target cell population BCG activated macro-phages selectively destroyed the neoplastic cells.

3. In addition to macrophage-induced tumor cytotoxicity as an index of effector immune responses, the mononuclear cell chemotactic assay was adapted for mouse peritoneal macrophages. Optimal culture conditions for the generation of complement and lymphocyte-derived chemotactic stimuli and for the macrophage responses to these stimuli were determined. The macrophage chemotactic response is now being utilized to quantitate yet another parameter of the effector immune response...the ability of macrophages to respond to defined levels of lymphocyte or complement derived chemotactic stimuli. Changes in macrophage chemotactic responsiveness have been observed in at least two situations: 1) agents which increase macrophage tumoricidal capacity in vivo and in vitro (BCG, endotoxin, polynucleotides, etc.) also increase macrophage chemotactic responsiveness. Agents which induce PE without inducing macrophage-mediated tumor cytotoxicity (oil, starch, thioglycollate, etc.) do not increase chemotactic responsiveness. These results suggest that the level of macrophage chemotactic responsiveness may reflect the ability of the animal to handle neoplastic challenge. Along these lines, the chemotactic responsiveness of peritoneal macrophages from tumor-bearing mice was analyzed. 2) the chemotactic response of peritoneal macrophages to both lymphocyte and complement derived chemotactic factors was depressed in tumor-bearing mice (methylcholanthrene-induced fibrosarcoma designated Tumor 1038). This depression was observed within 1 week after tumor injection (5×10^5 tumor cells into the footpad) and before the appearance of palpable tumor. Chemotactic responsiveness to both factors was about 50% below normal by 1-2 weeks and progressively decreased until the death of the animal by 6-8 weeks.

4. Chemistry of mouse lymphotoxin: killing of tumor target cells was measured by a chromium release assay. In contrast to other assays of lymphotoxin, this measures cell death rather than the total result of cell death and proliferation inhibition. With this assay, a killing factor from supernatants of mitogen-stimulated mouse spleen cells has been characterized by gel filtration and isoelectric focusing. It has a molecular weight around 50,000 and isoelectric focusing range of 4.5-5.0. Thus it appears greatly different from characteristics of mouse lymphotoxin reported previously (MW in the range 80,000-150,000 and a highly alkaline isoelectric point).

Significance to Biomedical Research and the Program of the Institute:

Analysis of the effector immune events leading to tumor rejection may provide a rational basis for manipulation of host responses to tumor cell in an effort to eliminate or prevent progression of the tumor.

Proposed Course of Project:

Studies on the mechanism of the killing of tumor cells by macrophages; on the factors modulating macrophage chemotaxis, especially in tumor-bearing mice; and further characterization of mouse lymphotoxin.

Publications

Boetcher, D. A. and Meltzer, M. S.: Mouse mononuclear cell chemotaxis: description of system. J. Natl. Cancer Inst, (In Press).

Cleveland, R. P., Meltzer, M. S., and Zbar, B.: Tumor cytotoxicity in vitro by macrophages from mice infected with Mycobacterium bovis, strain BCG. J. Natl. Cancer Inst. 52: 1887-1895, 1974.

Meltzer, M. S. and Cohen, B. E.: Tumor suppression by Mycobacterium bovis (strain BCG) enhanced by vitamin A. J. Natl. Cancer Inst. 53: 585-587, 1974.

Meltzer, M. S., Jones E. E., and Boetcher, D. A.: Increased chemotactic response of macrophages from BCG-infected mice. Cell. Immunol., (In Press).

Meltzer, M. S., Tucker, R. W., and Breuer, A. C.: Interaction of BCG-activated macrophages with neoplastic and nonneoplastic cell lines in vitro: cinemicrographic analysis. Cell. Immunol., (In Press).

Meltzer, M. S., Tucker, R. W., Sanford, K. K., and Leonard, E. J.: Interaction of BCG-activated macrophages with neoplastic and non-neoplastic cell lines in vitro: quantitation of the cytotoxic reaction by release of ^{3}H-thymidine from prelabeled target cells. J. Natl. Cancer Inst., (In Press).

Zwilling, B. S., Meltzer, M. S., and Evans, C. E.: Differential cytotoxicity of tumorigenic and non-tumorigenic strain-2 guinea pig cells mediated by syngeneic phytohemagglutinin stimulated peritoneal exudate cells. J. Natl. Cancer Inst., (In Press).

Project No. Z01-CP-04677-03-RGY
1. Biology Branch, Carcinogenesis, DCCP
2. Tumor Antigen Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mononuclear Cell Chemotaxis

Previous Serial Number: Same

Principal Investigators: Edward J. Leonard, M.D., David A. Boetcher, M.D., and Michael Lett-Brown, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	1.0
Professional	:	1.0
Other	:	0.0

Project Description

Objectives:

To develop quantitative measures of reactions occurring in the effector limb of the immune response; to study chemotaxis of inflammatory cells in relation to this objective.

Methods Employed:

Complement-derived and lymphocyte derived chemotactic factors were generated. Peripheral blood leukocytes were separated on Ficoll-Hypaque gradients. Standard methods of protein fractionation were employed to characterize chemotactic factors.

Major Findings:

Under certain conditions, delayed cutaneous hypersensitivity reactions have large accumulations of basophils. This is in contrast to the tuberculin reaction in which the predominant inflammatory cell is the macrophage. To elucidate the chemical basis of the difference in cell infiltrates, mitogen or antigen induced lymphokines were analyzed for human basophil or monocyte chemotactic activity. The two activities are remarkably similar, showing for example 2 distinct peaks on DEAE chromatography, both of which have both monocyte and basophil chemotactic activity. One fact favoring a

chemical difference in the 2 activities is that basophil chemotactic factor is consistently less stable than the monocyte factor. It was shown last year that supernatants from stimulated lymphocyte cultures greatly increase the responsiveness of human basophils to complement derived chemotactic factor. Recent experiments show that this augmentation factor is chemically distinct from lymphocyte-derived basophil chemotactic factor.

Significance to Biomedical Research and the Program of the Institute:

The monocyte chemotactic response (MCR) test is a useful measure of one function of the effector arm of the immune response. A defect of the effector system has been demonstrated in many cancer patients which may occur early in the progress of malignancy, affording opportunities for tumor detection, and may relate to patient prognosis. Clarifying the role of the effector arm of the immune response is essential for understanding the function of the immune system in tumor rejection. Studies on basophil chemotaxis may contribute to understanding the role of basophils in cellular immune reactions.

Proposed Course of Project:

Study of mechanisms of decreased MCR in cancer patients. Longitudinal study of abnormal chemotaxis in cancer patients. Further study of mechanisms of basophil chemotactic response. Studies on the role of basophils in immune reactions to tumor-specific transplantation antigens.

SUMMARY REPORT

CARCINOGEN BIOASSAY AND PROGRAM RESOURCES BRANCH

July 1, 1974 through June 30, 1975

"Plans, develops and implements a coordinated research program to (1) test chemical and physical agents for carcinogenicity by standard bioassay methods; (2) establish the necessary methods and logistics for the bioassay program; (3) develop and provide animal, chemical and information resources and data analysis to the Carcinogenesis Program; (4) monitor animal care and welfare in the intramural and collaborative contract programs; (5) develop and standardize selected animal models for carcinogenesis research; and (6) conduct studies aimed at determining the co-carcinogenicity of radiation with chemical agents."

The Branch has now completed its first full year in this status, having been elevated from a Unit in April 1973. Two personnel changes occurred during the year: (a) Dr. Cipriano Cueto was employed as Pharmacologist in November 1974 and (b) Mr. Terrance Kuch on loan by the Field Studies and Statistics Program for systems support, left the Branch and was not replaced.

While the Branch provides support to the entire Carcinogenesis Program, it has major responsibilities in two segments of the collaborative program: the Bioassay Operations Segment and the Information and Resources Segment. In addition, members of the staff also serve as project officers on contracts in the Biological Models Segment and Lung Cancer Segment. A discussion of these projects can be found in the segment reports.

While the Branch is not formally divided in sections, the activities of the Branch are related primarily to three main subjects as reported herein.

(1) Carcinogen Bioassay - Specific aspects of this activity relate to the (a) identification and selection of chemical and physical agents for bioassay; (b) establishment of logistical capabilities for testing; (c) acquisition, characterization, and purification of these agents; (d) identification, development and selection of improved animal systems and short-term bioassay procedures; (e) identification of carcinogenic activity of selected agents by the appropriate bioassay tests; (f) monitoring testing progress and performance; (g) development of a data bank to include results of bioassay testing and information on use and characterization of the agents being tested; (h) analysis and evaluation of the test results; and (j) decision on further action required of the tested agents. Approximately 540 chemicals have been selected and are now under test. The bioassays are conducted through contracts within the Bioassay Operations Segment. Members of the Branch provide management to those studies as well as support in animal/chemical and information/data areas. The main categories of compounds on test

are pharmaceuticals, industrial chemicals, pesticides and other agricultural chemicals, natural plant products, food additives and metallic compounds. Approximately 225,000 animals, mostly rats and mice have been used during the past year.

Guidelines for Carcinogen Bioassay in Small Rodents have been developed to document the procedures and methods used by the bioassay program to screen environmental chemicals for carcinogenicity through their oral administration to small rodents. They may also be applicable, in part or in toto, to other long-term studies using different procedures or with other objectives or endpoints.

The Guidelines have been submitted to reviews from academic institutions, trade associations, and other Federal agencies. This document describes many of the features which are common to all well-designed and properly conducted long-term animal studies, that need to be considered whenever a carcinogen bioassay is planned or undertaken.

The Guidelines have been made available as an administrative document and plans are underway to submit them for publication in a national scientific journal and also in the NCI Technical Report Series. It is planned that as new sections are developed and changes are recommended, these Guidelines will be appropriately revised. Branch staff have worked closely with Doctor J. Sontag, Manager, Bioassay Operations Segment, to develop these Guidelines.

The establishment of a Bioassay Operations Prime Contract (Tracor Jitco, Inc.) has led to substantial savings of cost and labor/time. The prime contract has relieved the Branch staff, still limited to only four full-time professionals, of many routine tasks allowing them to devote more of their time to the scientific development of the program.

Procedures in selecting chemicals for carcinogenesis bioassay were further developed during the past year. The Prime Contractor now provides support to the Chemical Selection Working Group. Nominations of chemicals for bioassay from other Federal agencies were greatly accelerated primarily through the efforts of the Scientific Coordinator for Environmental Cancer. The main criteria used for selection are exposure/production data and chemical structure.

The Branch is responsible for the preparation of critical reviews of the results of bioassay testing. This requires evaluating the conduct of the experiments, and analyzing the results with assistance from the Field Studies and Statistics Program. Since the results may be important in establishing public policy on the use of the agents tested, release of information must be made following a critical evaluation of the results and with the approval of the Associate Director for Carcinogenesis. The Scientific Coordinator for Environmental Cancer coordinates release of information to other Government agencies.

One of the most pressing concerns in the bioassay program is a need to fully document and make available the details of the methodology, conduct

and results of each chemical bioassay. While usual reports in open literature or conferences will still be encouraged, a Technical Report Series has now been established. While the Prime Contractor will provide most of the effort in preparing the Technical Reports, staff personnel will contribute to assure input of data from the Carcinogenesis Bioassay Data System (CBDS) and from various laboratories, establish the priority for preparation, and coordinate the formal review and approval of the reports, and their publication and distribution by the Government Printing Office. As the number of chemicals completing test is now reaching its peak, this represents a major undertaking.

The CBDS is used to collect, monitor and store experimental data and results from bioassay operations. It is now in full operation with new bioassay experiments being entered as they are initiated. Data from 20 contractors are now on file with several other contractors preparing data for entry into the system. Considerable progress has been made in entering data into the CBDS. During this past year attention has been directed to the formatting and generation of data tables and reports both for management of ongoing studies as well as inclusion in the Technical Reports now being developed. In addition, chemical and bioassay contract information sub-systems are maintained which list data on materials presently under test or projected for study, date initiated, etc.

During this past year the Branch staff have convened or participated in several workshops or meetings on problems of methodology and interpretation of carcinogenesis bioassays with emphasis on the study of environmental chemicals of public interest. A particularly important workshop was held on January 29-30, 1975, to discuss the current NCI pesticide carcinogenesis studies and the design and implementation of a bioassay protocol for more in-depth studies of the chlorinated hydrocarbon pesticides.

The Branch organized a workshop on inorganic arsenic on March 19, 1975, to discuss carcinogenesis research needs and coodinate efforts underway by the Federal agencies. Support has been provided to the Scientific Coordinator for Environmental Cancer in meetings on rotenone and carcinogenic contaminants in water. The Branch Chief, Dr. N. Page, has served as Chairman of an Interagency Subtask Group on Carcinogen Testing, Interagency Collaborative Group on Environmental Carcinogenesis, which is reviewing protocols or guidelines in use by various Federal agencies, and as NCI representative on a task force to study the capabilities in the Government in inhalation carcinogenesis studies and define role that the National Center for Toxicological Research might play in this regard.

(2) Information Activities - These are devoted to (a) develop, implement and maintain information systems to gather, catalogue, and disseminate information to the Carcinogenesis Program's scientific and administrative staff and (b) perform literature searches and compile information as a staff support activity.

The Branch staff contribute considerable effort to the publication of the Survey of Compounds Which Have Been Tested for Carcinogenic Activity (PHS Publication No. 149) and Carcinogenesis Abstracts. Editorial review and the distribution of mailing keys are maintained by the Branch.

Carcinogenesis information searches were conducted as requested by Carcinogenesis staff utilizing data bases such as the Smithsonian Information Exchange, MEDLARS, National Library of Medicine's Toxicology Information Program, Selective Dissemination System of the USDA and Environmental Mutagen Information Center (EMIC). Branch staff collaborated with the NCI's Office of the Director in the development of the International Cancer Research Data Bank and the NCI Management Information System.

(3) Animal Resources and Care - Specific aspects of this activity relate to (a) procurement under contract with Information and Resources Segment of animals for the intramural and collaborative contract programs; (b) assurance of compliance with the animal welfare laws and HEW Guide for Care and Use of Laboratory Animals; (c) assistance to the Carcinogenesis staff with animal health and husbandry problems and plans animal facility modifications; and (d) contributions to the development of animal models for cancer research as well as to the collection and dissemination of baseline information on laboratory animals.

The Branch assists the program directors in planning their animal needs as far in advance as possible, coordinating these needs with the animal suppliers and providing information on the characteristics and availability of laboratory animals. Assurances of compliance with the Animal Care and Welfare standards are obtained for both contract and intramural programs.

Doctors Page and T. Cameron have been instrumental in the NCI's effort to improve animal care in the intramural and collaborative contract programs. Doctor Page relinquished the Chairmanship of the NCI Ad Hoc Group on Animal Care and Welfare to Doctor Cameron.

(4) Other Activities - The Branch Chief functions as the radiation biologist for the Carcinogenesis Program. A small program on radiation/chemical interactions is underway, supported by contracts in the Biological Models and Lung Cancer Segments.

Project No. Z01-CP-04539-04-CPBR
1. Carcinogen Bioassay and Program Resources Branch, Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Coordination of Information Activities and Data and Information Evaluation and Dissemination for the Carcinogenesis Program

Previous Serial Number: Same

Principal Investigator: Sidney Siegel, Ph.D.

Other Investigators: Norbert P. Page, D.V.M.; Umberto Saffiotti, M.D.; John A. Cooper, Ph.D.; Thomas P. Cameron, D.V.M.; Marcia D. Litwack, Ph.D.

Cooperating Units: None

Man Years:
Total : 2.0
Professional: 1.5
Other : 0.5

Project Description

Objectives:

To develop, implement and maintain systems which gather, catalogue, disseminate and evaluate data and information relevant to the Carcinogenesis Program.

By creating, maintaining and/or searching data bases which will enable scientists and scientist administrators, within the articulation of disciplines comprising carcinogenesis research, to be made aware of data and information relevant to their own efforts and responsibilities.

In so doing, standardize the approaches to data and information accumulation, storage, retrieval and evaluation to increase the effectiveness and economy of the various information systems and activities.

Methods Employed:

While much of the data and information gathering and dissemination is performed by Branch personnel, the needs far exceed in-house capability, and contractors are employed to perform specific tasks. A full listing of these tasks is available in the section describing the Information and Resources Segment. Activities used to respond to program needs can be generally grouped as regularly occurring publications which gather abstract and/or extract relevant data and information from the scientific literature or as specialized information projects ordered to meet special intra- and/or extramural research and/or administrative needs. These information projects, in various stages of evolution, have enabled Program to logically explore and also establish the feasibility of the various approaches needed to define, uncover, collate, store, retrieve and evaluate data and information on research, research-administrative and regulatory responses to problems which exist now and are projected for the Carcinogenesis Program.

Coordination of the existing, experimental and projected nodes of information activity is required for efficient, effective and economical responses to Program's information problems. Among these problems are concerns of the interpretation of the relationship of the toxicologic phenomena exhibited by biologic systems and their responses to agents which are carcinogens, mutagens, teratogens and further concerns as to how these chemical agents are distributed and impact on the human population and the environment.

Major Findings:

Literature extraction activity required of the contractor for Carcinogenesis Abstracts; the PHS-149, Survey of Compounds Which Have Been Tested for Carcinogenic Activity and the Mutagenesis Data Base, indicated that there has been a significant and stable increase of program relevant information from the various disciplines which comprise carcinogenesis research.

Significance to Biomedical Research and the Program of the Institute:

Development, implementation and monitoring of research programs requires a continual flow of information, which must be ordered or indexed in a manner to allow the data and information generated to be searched or manipulated in logical and useful ways. Once data bases are established, criteria on reliability of new data can be used as a qualification filter for inclusion or as a method for annotating what is retained. Information projects which are now on-going and those ready for implementation are designed to complement scientific and scientific-administrative decision making needs of the Carcinogenesis Program.

Proposed Course of the Project:

The project will continue its formal information publications as in the past and these as needed will be modified as to content, format and media to increase their utility.

Program is exploring the methodology of developing, on a modular basis, the capability of disseminating data generated by or of interest to carcinogenesis researchers and administrators.

The project will continue to be sensitive to the general and specific information needs of the Carcinogenesis Program. Existing information systems and those to be generated are and will be so structured that as to be able to respond to the ever-changing profile of research. The systems are designed to store new and different data with more conventionally formatted data of the past while still having the ability to search and retrieve both kinds of information and will have the capability of helping other communities, federal, state, academic, industrial and concerned citizens, meet their data and information needs.

Honors and Awards

Member, Information and Resources Segment

Member, Bioassay Operation Segment

Member, Interagency Collaborative Group on Environmental Carcinogenesis NCI.

Member, Toxicology Information Subcommittee of the DHEW Committee to Coordinate toxicology and Related Programs.

Member, Task Force on Formulation of a Control Animal Data Bank which will reside in the National Library of Medicine's Toxline System, NIH

Member, Task Force on Toxicology Research Information Depository at the National Technical Information Service, U. S. Department of Commerce

Project No. Z01-CP-04540-04-CPBR
1. Carcinogen Bioassay and Program Resources Branch, Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Radiation Carcinogenesis Program

Previous Serial Number: Same

Principal Investigator: Norbert P. Page, D.V.M.

Other Investigators: D. Jane Taylor, Ph.D., Head, Experimental Biology Project Section, DCBD

Cooperating Units: Energy Research and Development Agency (ERDA/AEC); Bureau of Radiological Health, FDA, HEW

Man Years:
Total : .30
Professional: .20
Other : .10

Project Description

Objectives:

(1) To assess interactions and synergism of radiation and chemical agents in the induction of breast and lung cancer. (2) To determine the effects of increased UV radiation from depletion of stratospheric ozone on skin carcinogenesis.

Methods Employed:

Much of this Program is devoted to the study of interactions of radiation with other environmental agents. A considerable effort is made to avoid duplication of research underway by other agencies.

To remain informed of research conducted by other agencies, progress reports or proposals of other agencies pertaining to on-going or proposed research programs in radiation carcinogenesis are reviewed. This area has been developed mainly through the collaborative contract program due to staff and research laboratory limitations with the intramural program.

To assess for interactions or synergism, groups of animals are exposed to radiation and chemicals alone, simultaneously or sequentially, and maintained for their normal lifespan to compare the relative incidence and type of tumors induced. The lung cancer studies use particles of alpha-emitting Polonium-210 and/or Benzpyrene-hematite. The interaction of smoking and radiation is assessed by epidemiological and exfoliative cytology studies of uranium miners. Estrogenic hormones, and radiation (X and neutron) are used to study interactions in the induction of breast cancer.

To assess the effects of depletion of stratospheric ozone on UV-induced skin carcinogenesis, an ozone chamber has been constructed to produce various thicknesses of ozone that filter the solar-simulated UV spectrum. Hairless mice are exposed to the UV radiation and maintained for the development of skin tumors.

Major Findings:

Summaries of on-going radiation carcinogenesis research have been obtained through the Science Information Exchange (SIE) of the Smithsonian Institute. Contracts have been made with other Government agencies to receive annual progress reports as well as other information pertaining to radiation carcinogenesis research. A close liaison has been established with the ERDA/AEC in this regard.

While much research has been conducted on the hazards of radiation, the mechanisms by which radiation exposure can result in cancer and of the interactions of radiation with other agents remains largely unknown. The results from these studies indicate that radiation acts synergistically with chemicals in inducing lung cancer in animals. From the uranium-miner studies a similar relationship appears to exist in man. A similar synergism has been found for breast cancer in both X and neutron radiation with hormones in rats. However, a strain difference in synergism and induction of breast cancer has been found.

Significance to Biomedical Research and the Program of the Institute:

The National Cancer Institute is concerned with carcinogenesis regardless of the etiological agent. Within the past few years the Institute has not supported a large program in radiation carcinogenesis mainly due to the commitment of other agencies in this regard. The mechanisms by which environmental agents induce cancer may be similar for all etiological agents. Radiation represents a research tool which is easy to use compared with chemical or biological agents. Understanding radiation carcinogenesis may be important in developing an understanding of carcinogenesis induced by other etiological agents. From the hazards viewpoint, radionuclides have been demonstrated to be a potent class of carcinogens and represents a risk to mankind that must be adequately evaluated. Preliminary results of current programs of the Carcinogenesis Program would indicate that in certain cases radiation and chemical agents

can act synergistically to induce cancer. The expertise available in the National Cancer Institute's intramural and contract programs can provide important direction in the assessment of radiation hazard. With the introduction of new materials, for example, the neutron-emitter Californium-252 as an isotope useful in biomedical research and industrial applications, additional research is required to assess their potential hazards.

It is important that the NCI assess the possible effects of depletion of the stratospheric ozone layer by supersonic transports or chemical interactions, e.g., freons on UV-induced skin cancer.

Proposed Course of Project:

It is expected that liaison and exchange of information with other agencies can be continued during the coming year. The major studies assessing the interaction of x-radiation and estrogenic hormones in inducing breast cancer and the interactions of alpha radiation and benzpyrene in inducing lung cancer have demonstrated synergism. Studies are now underway to determine the mechanisms involved. Preliminary results have demonstrated that neutron-radiation and hormones also interact in a synergistic manner in inducing breast cancer. The contract to study UV-radiation induced skin carcinogenesis should reveal results within the next year.

Honors and Awards

Chief, Carcinogen Bioassay and Program Resources Branch

Director, Bioassay Operations Segment, Carcinogenesis, NCI

Member, Division of Cancer Cause and Prevention Interprogram Group, NCI

Member, Carcinogenesis Contract Program Management Group

Member, Interagency Collaborative Group on Environmental Carcinogenesis

Member, Information and Resources Segment, Carcinogenesis, NCI

Chairman, Interagency Sub-task Group on Carcinogen Testing

Member, Chemical Selection Working Group, Carcinogenesis, NCI

Member, Experimental Design Group, Carcinogenesis, NCI

Project No. Z01-CP-04541-04-CPBR
1. Carcinogen Bioassay and Program Resources Branch, Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Animal Resources

Previous Serial Number: Same

Principal Investigator: Thomas P. Cameron, D.V.M.

Other Investigators: Norbert P. Page, D.V.M.

Cooperating Units: Animal Farm, Frederick Cancer Research Center; Carl Hansen, Ph.D. Genetics Unit, Rodent and Rabbit Production Section, VRB, DRS; Joseph Mayo, D.V.M. Mammalian Genetics and Animal Production Section, DCT, Charles River Breeding Laboratory

Man Years:
Total : 4.3
Professional: 1.3
Other : 3.0

Project Description

Objectives:

(1) Plan and effect Procurement of animals used in the Carcinogenesis intramural and contract programs; (2) Assure compliance with the Animal Welfare Laws and the DHEW Guide for Care and Use of Laboratory Animals; (3) Plan and support the development of animal models for cancer research as well as to collect and provide information on laboratory animals; and (4) Provide general guidance on facility construction renovations, and methodology to contractors.

Methods Employed:

The number of animals required for the various aspects of the Carcinogenesis Program are estimated as far in advance as possible by the program staff, staff of the Bioassay Program Prime Contractor, and the staffs of the various contracts.

The bulk of these needs are met by the Animal Farm at FCRC; the breeding stock for that effort continues to be supplied by the Genetics Unit of the Small Animal Section, VRB, DRS. A modest number of animals of varying species, strains and stock continue to be supplied by the Mammalian Genetics and Animal Production Section, DCT under the mechanism of fund transferral from the Carcinogenesis Program to DCT.

The system of projecting, ordering and reporting animal deliveries of the various species and strains, formerly in operation between Wolf/EG&G and the production staff of the Animal Farm, FCRC, has been modified. The Prime Contractor for the Bioassay Program, Tracor Jitco, is now responsible for transmitting the animal needs of their subcontractors to the FCRC staff. The demands of other contractors and intramural staff are still coordinated through the Carcinogen Bioassay and Program Resources Branch to FCRC.

Staff members continue to be involved in the animal resource and welfare aspects of the Bioassay Program, cooperating with and training, when necessary, the Prime Contractor's staff.

Two task orders have been attached to the NIH contract with the Institute of Laboratory Animal Resources, National Research Council/National Academy of Sciences. The initial order, the development of a publication, "Standards for Successful Chronic Rodent Studies" (Mice, Rats, and Hamsters), is being prepared now. This document will serve to bring a measure of standardization of facilities, equipment, and methodology to the present and prospective contractors of the collaborative program and could serve as a useful guide for the grants program.

The second order assigned was the preparation of a document concerning the "Athymic" or Nude Mouse. It will be a "state of the art" summary concerning the known genetics, husbandry and uses of this unique immunologically incompetent animal that promises to become a major animal resource in cancer research. This is not designed to be a final pronouncement on this special strain and as additional information on this animal is authenticated, it may well be added to the original document in whole or part as desirable. Progress on this task order has been exceptionally swift and it is expected to be ready for publication by the end of this contract year.

Contract support has been continued to Dr. Ulrich Mohr, Medizinische Hochschule, Hannover, West Germany for the development of the European hamsters as an animal model primarily for use in lung cancer research. Very preliminary information indicates that this hamster may offer increased value as a model for pancreatic tumor induction. A breeding colony, to our knowledge the first in the U. S., has been established at the NIH Animal Center at Poolesville, The nucleus of some twenty-eight (28) animals was obtained from Dr. Mohr. The animals are being acclimated and it is anticipated that they will begin breeding in Spring, 1975.

A production effort contract was initiated this year at the Charles River Breeding Laboratory, Wilmington, Massachusetts, to produce B6C3F1 mice and F344 rats to be utilized mainly in the Bioassay Program. The initial year's effort will be devoted to isolator operations, either as germ-free or with an associated flora. The barrier supported production component is expected to commence in mid-year FY 1976. This contract is intended to supplement the production of the Animal Farm at FCRC and provide "insurance" for the animal resource of the Bioassay Program. As with the FCRC colonies, the initial breeders and any subsequent nucleus breeders are and will be provided by the NIH inhouse colony.

Permission was obtained to establish a source of aged male rats, virgin and experienced breeders, of one strain, the ACI, and the Sprague Dawley stock. At the present time, proposals have been received and entered into the evaluation process. Awarding a single or multiple contract will be accomplished during this contract year. The product of this effort will be used to support the prostate cancer program with distribution of the animals directly under staff supervision.

Through staff efforts, a highly qualified individual in laboratory animal science was recruited for the staff of the Institute's Director. Close liasion has been established with this individual and a seminar on "Biohazard Aspects of Primate Experimentation" was jointly organized and presented at FCRC.

The introduction of ectromelia into Building 37 was successfully confined under the direction of staff and with the fullest cooperation of the staff of NCI and other components of the NIH. At the present, staff members are part of an effort to write a procedures manual that will be of value in preventing future episodes of this nature by establishing admission and quarantine regulations.

Major Findings:

The major effort of this resource area has been to insure the production of and proper distribution of sufficient numbers of animals of extremely high health status for the efficient functioning of the Bioassay Program. The production level at the FCRC Animal Fram is now peaking at a rate - 10,000/F344 rats/month and 12,000/B6C3F1 mice/month that will more than satisfy the combined needs of DCCP and the DCT chemotherapy program. An additional production effort has been initiated at the Charles River Breeding Laboratories. With the established germ-free nucleus and a gnotobiote expansion unit also maintained in isolators, this back-up to the FCRC should reach its assigned production level of 1000/F344 rats/ month and 1000/B6C3F1 mice/month by mid FY 76.

The strain 2 guinea pig colony is currently just meeting the demands made upon it while the strain 13 colony issues must be made on program priority basis. The insufficiency of strain 13 pigs has developed during this past year as a direct result of the collaborative program's experimental findings that strain 13 male weanlings offer the promise of developing into a pancreatic tumor model. This is an exciting advance and one that puts extraordinary demands upon a strain that inherently has an extremely low reproductive efficiency index - six (6) weanlings/sow/year. All possible measures are being taken to increase both the strain 2 and strain 13 colonies to the limit of the space available.

The contract to develop the European hamsters as an animal model continues to be productive. A paper, presentingthe early detection of induced lung tumors by x-ray, was given at the National meeting of the American Association for Laboratory Animal Science. The staff of the contract has assembled an excellent English language anatomical atlas of this animal and it will be edited under our auspices for possible publication before the end of this fiscal year.

The nucleus of a breeding colony, twenty eight (28) animals has been established at the NIH Animal Center. It is anticipated that these animals will begin breeding in the spring of 1975 and that sometime in the beginning of 1976 animals can be made available for initial experiments.

Significance to Biomedical Research and the Program of the Institute:

Biomedical research, to a very large degree, depends upon the selection of the proper animal model, the supply of sufficient numbers of healthy animals to satisfy the protocols and the correct care of the the animals while under test. These factors are especially important to the extensive Bioassay Program in which animals are held for the greater part or all of their lifespan to study the carcinogenic influences of certain compounds. Animal deaths, from non-experimental influences midway or later in this type of study, represent a significant loss in time and money.

It is essential that the staff supply the proper quality and numbers of animals to the program and monitor their care both in the intramural and extramural programs. This is done through the contract mechanism (as project officers or resource advisors) for the extramural effort and as members of the NCI Ad Hoc Group on Animal Care and Welfare in the case of the intramural components.

Likewise, the development of new or more sensitive animal models in cancer research is a vital concern to program. As new animal needs are identified and demands are created, the supply mechanism must be created so that biomedical research progresses.

Proposed Course of the Project:

The project will continue as defined. The extramural, and to a more limited extent, the intramural programs will be serviced in the areas of animal supply, advice as to husbandry practices and the development of needed animal models in collaborative efforts. To this end, the contracts to establish sources for aged virgin and breeder males of one inbred stock will be awarded by the end of FY 1975.

As predicted, the input of staff time in the direct management of the animal aspects of the Bioassay Program has been somewhat lessened by the Prime Contractor. The time gained by this relief will be directed toward assisting in the establishment of a control animal data bank under the direction of biomedical information specialists. In addition, extra effort will be directed when requested to assist in upgrading animal husbandry practices in existing contracts and new awards of contract areas, other than the Bioassay Program, in which this aspect has traditionally been given a lower priority.

Honors and Awards

Chairman, NCI Ad Hoc Group on Animal Care and Welfare

Program Chairman, 25th Annual Session of the American Association for Laboratory Animal Science

Publications

Cameron, T. P., Kasel, J. A. and Couch, R. B.: Persistence of Antibody to Envelop Antigens of Heq2Neq2 Virus in Ponies after Infection and Vaccination (38166). Proc. Soc. Exp. Biol. Med. 146: 658-660, 1974

Project No. Z01-CP-04564-02-CPBR
1. Carcinogen Bioassay and Program Resources Branch, Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Carcinogen Bioassay Program

Previous Serial Number: Same

Principal Investigator: Norbert P. Page, D.V.M.

Other Investigators: James M. Sontag, Ph.D.; Cipriano Cueto, Jr., Ph.D. Thomas P. Cameron, D.V.M.; Robert A. Squire, D.V.M., Ph.D.; Elizabeth K. Weisburger, Ph.D.; John A. Cooper, Ph.D.; Umberto Saffiotti, M.D.; Herman F. Kraybill, Ph.D.; Sidney Siegel, Ph.D.; Marcia D. Litwack, Ph.D.; Patricia A. Steinour; John J. Gart, Ph.D.; and Thomas R. Fears, Ph.D.

Cooperating Units: Collaborating Scientists and Advisors of the Carcinogenesis Program; Field Studies and Statistics Program; Tracor Jitco, Incorporated; Wolf Research and Development Corporation

Man Years:
Total : 4.5
Professional: 3.0
Other : 1.5

Project Description

Objectives:

To coordinate and manage the Carcinogen Bioassay Program which identifies and evaluates carcinogenic hazards of chemical and physical agents in the environment.

Methods Employed:

Standardized long-term animal bioassay tests are utilized to assess for carcinogenicity. Considerable planning is required to procure and coordinate the animal, chemical, information and laboratory resources required for an efficient and quality operation. Details of specific methods employed are provided in "Project Reports" for the particular activity.

As resources and funds are not available to test all chemicals introduced into the environment, a judicious selection must be made. A Chemical Selection Working Group selects chemicals on the basis of human exposure, production data, and chemical structure. Detailed dossiers for each chemical considered are prepared. Nominations of chemicals come from various sources including other Federal agencies. Once selected, the chemicals must be procured and analyzed. The majority of testing is by contract with commercial laboratories or special research centers having the required facilities and scientific staff.

In addition to providing for testing facilities and chemical resources, animal needs must be forecast well in advance. The main animals used in the Bioassay Program are the Fischer/344 and Osborne-Mendel rats and B6C3F1 hybrid mouse (C57B1/6 x C3H). Most of the animals are supplied by the Frederick Cancer Research Center. Animals on long-term tests must be maintained under strict environmental surveillance to prevent the introduction and spread of infectious diseases that can quickly decimate rodent colonies.

The chronic testing must be carefully planned to assure that the proper experimental design is used and that the bioassay is conducted under optimal conditions. Monitoring of the quality and progress of individual studies are through visits and reviews, the Carcinogenesis Bioassay Data System (CBDS), and routine progress reports.

It is important that bioassay data be recorded, analyzed and reported properly. Currently, this is through two main means. For studies initiated in the past 2 1/2 years, data are retrieved by use of a computerized data system known as the Carcinogenesis Bioassay Data System (CBDS). This is described in Individual Project Report NCI-4699.

Contractors are encouraged to submit the results of their testing to scientific journals having wide dissemination to the scientific community. As journals often will not accept bioassay data due to the bulk and often negative results, a decision to implement a Technical Report Series has been made and is now being implemented.

It is important that the results of these tests be properly tabulated and analyzed prior to release to the public or other agencies. For this reason all release of bioassay data must be reviewed and approved prior to contractor release. The Scientific Coordinator for Environmental Cancer is responsible for discussing the results with other agencies. In the event that interim results indicate that a carcinogenic response has been demonstrated, a preliminary note or "memorandum of alert" may be released for chemicals to which there is considerable human exposure.

In March 1974 Tracor Jitco, Incorporated was awarded a Prime Contract for the Bioassay Program. Ten of the prior NCI contracts are now subcontracts. The Prime Contract provides administrative and scientific management support to the bioassay operations.

Major Findings:

A large expansion of the program took place in the past two years; such that approximately 540 chemicals were on test in FY 1975. This includes a large number of pharmaceutical agents, pesticides and other agricultural chemicals and industrial chemicals to which man is exposed. A number of these chemicals, especially pharmaceuticals and pesticides, have been found to possess strong carcinogenic activity. During this current year several other long-term experiments have terminated and are awaiting completion of pathological examination and data analysis.

After considerable review and revisions the methods used in the carcinogenesis testing program have been modified and documented in "Guidelines for Carcinogen Bioassay in Small Rodents." These Guidelines have now been incorporated into the testing program of other Federal agencies.

The use of a Prime Contract for biomedical research appears to be a feasible method of administering carcinogenesis testing studies and has relieved the Carcinogenesis staff of many routine duties allowing them to pursue more scientific aspects of the Program.

Significance to Biomedical Research and the Program of the Institute:

Protecting the public from chemical and physical carcinogenic hazards and their effects has been clearly recognized as an urgent and essential task in our society and a high national priority for this mission has been indicated by the National Cancer Act, Congress and the Administration. One of the major program activities is the identification of carcinogenic activity of selected agents by bioassay testing. The results of this testing program are utilized in the establishment of public policy by regulatory agencies in regard to utilization of the test agents. It is estimated that several hundred thousand different chemical entities are present in the environment. With modern technology the annual accrual rate is increasing the environmental burden at an astounding rate. Many of these factors have not yet been assayed for carcinogenicity. It is important that such information be obtained if the current epidemic of cancer is to be abated. There is little doubt that environmental agents play a major role in the causation of cancer. It is anticipated that the results from this program may prevent the unintentional exposure of man to carcinogenic materials.

Proposed Course of Project:

The expansion of the program was temporarily halted during the past year due to staff and resource limitations. The resources are now capable of

meeting the anticipated needs of the program. Additional agents have been identified by the Chemical Selection Working Group. While budgetary restraints will not permit all those of high priority to be tested; several have gone on test this past year. The Prime Contract has helped resolve much of the staff problems.

Many chemicals have reached the end of their testing phase resulting in a heavy workload in pathology, data analysis and reporting.

Additional studies are needed to improve the methodology of testing. Research is needed to better predict a tolerated level for long-term treatment. One of the major changes in the bioassay protocol was to extend the period for the subchronic range-finding tests. Additional studies are planned to assess species/strain sensitivity to allow better selection of the test animal.

A limiting factor in the test program is that of completing the extensive pathology studies now required by the revised "Guidelines." Methods are needed to streamline the pathology aspects for maximum utilization of the pathologist's time.

There is an urgent need to standardize the conduct of pathology techniques and reporting. The SNOP-code was developed for human pathology and needs refined for use with animal experimental results.

To effectively manage and monitor the Bioassay Program requires expertise in various disciplines including research management, animal care, chemistry, toxicology, biometry, pathology, information handling, and fiscal operations.

While the Carcinogen Bioassay and Program Resources Branch, cooperating groups in Carcinogenesis, Prime Contract and support pathology and information contracts possess most of these disciplines, the need for more intramural staff is urgent in view of the large workload and importance of the research.

Honors and Awards

Chief, Carcinogen Bioassay and Program Resources Branch

Director, Bioassay Operations Segment, Carcinogenesis, NCI

Member, Division of Cancer Cause and Prevention Interprogram Group, NCI

Member, Carcinogenesis Contract Program Management Group

Member, Interagency Collaborative Group on Environmental Carcinogenesis

Member, Information and Resources Segment, Carcinogenesis, NCI

Chairman, Interagency Sub-task Group on Carcinogen Testing

Member, Chemical Selection Working Group, Carcinogenesis, NCI

Member, Experimental Design Group, Carcinogenesis, NCI

Publications

Althoff, J., Mohr, U., Page, N., and Reznik, G.: Carcinogenic Effect of Dibutylnitrosamine in European Hamsters (Cricetus cricetus). J. Natl. Cancer Inst. 53: 795-800, 1974.

Project No. Z01-CP-04699-07-CPBR
1. Carcinogen Bioassay and Program Resources Branch, Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Carcinogenesis Bioassay Data System

Previous Serial Number: Same

Principal Investigators: Norbert P. Page, D.V.M.; John A. Cooper, Ph.D.; Mary S. Linhart; Umberto Saffiotti, M.D.

Other Investigators: Sidney Siegel, Ph.D.; Ursula Evans

Cooperating Units: Data Management Branch, Division of Computer Research and Technology, NIH; Wolf Research and Development Corporation; Field Studies and Statistics Program, NCI

Man Years:
Total : 3.00
Professional: 2.00
Other : 1.00

Project Description

Objectives:

To develop, implement, and maintain a comprehensive information system for collection, storage, manipulation, retrieval, analysis and reporting of data pertaining to carcinogenesis bioassay studies. In so doing, to create and maintain a file of data about (a) substances on test in the Carcinogenesis Program, (b) experiments and related experimental design in bioassay studies, (c) diagnoses of animal deaths from suspected carcinogenic agents on trial, and corresponding control groups.

Methods Employed:

Standard taxonomic, system-operational, systems design, computer programming, and statistical computing techniques, as well as the NIH/DCRT hardware and software environment have been used in the development of the system.

A chemical subsystem is in operation and contains relevant data on substances under investigation. It can be used for quick response upon inquiry.

The Carcinogenesis Bioassay Data System comprises four basic subsystems which are described below:

(1) Bioassay Contractor Subsystem - The contractor scientists document the design and results of experiments on a series of standard forms to the NCI through the Operations Subsystem.

(2) Operations Subsystem - An operations contractor provides and obtains completed forms from the bioassay contractors, checks them for accuracy and completeness, logs their progress through the system, microfilms them, keypunches, key-verifies input documents corresponding to the data forms, and submits computer jobs on new or replacement data for entry into the CBDS data base. Also maintains auxilliary bioassay information files.

(3) Computer Subsystem - The computer systems manager and programmers design, implement, maintains the CBDS data base and the CBDS system of computer programs to manage that data base, and generates preliminary management and scientific output reports.

(4) Statistical Analysis Subsystem - Computer programmers and statisticians are teaming to implement statistical routines for analyzing chronic toxicity and carcinogenicity data of CBDS test animals.

Major Findings:

Many experiments have come to completion, allowing data from them to be fully analyzed in the statistical analysis subsystem. Computer Subsystems output, in the form of detailed summary reports and simple graphs, have been perfected during the past year. Maintenance will continue throughout the life of the project to ensure that the data system is compatible with divergent experimental designs.

Significance to Biomedical Research and the Program of the Institute:

Because of the public importance of the Carcinogenesis Bioassay Program, it is essential that data and administrative information be quickly and accurately available. The CBDS provides this capability and contributes uniformity and completeness to reporting results of bioassay studies and to standardization of chemical and tumor nomenclature.

Proposed Course of Project:

New bioassay studies will be entered into the system as they are initiated. During FY 1976, it is estimated that data reports will be generated on approximately 60 chemicals. Data from new and existing contractors will be entered into the CBDS. The contractor for the operations subsystem has proven responsive, functioned in an acceptable manner, and will continue as in the past. Maintenance of the computer subsystem will be continued using personnel from the DCRT. Preliminary analysis data reports are now being generated. Additional statistical analyses and methods of presenting data will be initiated.

Project No. Z01-CP-04699-07-CPBR

Honors and Awards

John A. Cooper II, Ph.D.

Director, Information and Resources Segment, Carcinogenesis, DCCP, NCI

Member, Cause and Prevention Executive Staff, DCCP, NCI

Member, Carcinogenesis Contract Program Management Group, DCCP, NCI

Member, Carcinogenesis Intramural Program Group, DCCP, NCI

Member, Chemical Selection Working Group, Carcinogenesis, DCCP, NCI

Publications

Linhart, M.S., Cooper, J., Martin, R.L., Page, N., and Peters, J.: Carcinogenesis Bioassay Data System. Comput. Biomed. Res. 7: 230-248, 1974.

SUMMARY REPORT

CARCINOGEN METABOLISM AND TOXICOLOGY BRANCH

July 1, 1974 through June 30, 1975

"Plans, develops, and conducts a research program including (1) identification, chemical analysis, mode of formation, and metabolism of various classes of chemical carcinogens; (2) studies on the toxicology and metabolic pathways of carcinogens in selected animal systems and in relation to man; (3) correlation of chemical and toxicological data for the selection and screening of chemical carcinogens; (4) development of biological and biochemical methods for the identification of reactive carcinogenic metabolites; and (5) metabolic activation of carcinogens by bacteria in the digestive tract."

The Carcinogen Metabolism and Toxicology Branch (CMT) is heavily involved in both inhouse research projects and in the scientific direction of collaborative projects through segments of the Carcinogenesis Program.

The collaborative projects are conducted through the contract segment which coordinates contracts on the toxicology and metabolism of various carcinogens and part of the program on nitrosamines. The staff of the Branch are also involved in the Information and Resources Segment, especially the remaining projects on nitrosamines and the repository of chemical carcinogens. The staff of the Bacterial Metabolism Unit of the Branch is largely responsible for the activity of the Colon Cancer Segment. Branch staff are also involved in selection of compounds for the bioassay program and acting as monitors for certain portions of the bioassay projects. Through the CMT Branch various contracts on development and conduct of short term bioassay systems as prescreens for carcinogens based on the mutagenicity of bacteria and mammalian cells and on either in vitro or host mediated assays have been conducted.

The inhouse activities of the Branch members are directed largely toward studies on the metabolism of various classes of chemical carcinogens, improvement of bioassays for such compounds, and studies on the metabolic activation of carcinogens by bacteria. They are described in the following paragraphs.

Metabolic Studies Section - *"Plans and conducts research including (1) identification, mode of formation and metabolism of various classes of chemical carcinogens; (2) studies on the toxicology and metabolic pathways of carcinogens in selected animal systems and in relation to man; and (3) correlation of chemical and toxicological data for the selection and screening of chemical carcinogens."* During the past year this Section has concentrated heavily on the metabolism of the environmental material 2,4-toluenediamine, an intermediate for polyurethane foams. A fair number of possible metabolic intermediates of the compound have been worked out. Both in vivo and in vitro studies with this compound have been performed. The in vitro studies, carried out with liver

cytosols from various species, showed that N-acetylation was a primary path of detoxification of 2,4-toluenediamine. There were appreciable differences in the rate of N-acetylation of liver cytosols from different species. A partial explanation for the resistance of mice to any effects of this compound may lie in the fact that they N-acetylate toluenediamine at a much greater rate than rats. The in vivo metabolic studies showed that a very high proportion of the material was excreted by rats within 24 hours of a single intraperitoneal dose. The major unconjugated urinary metabolites were some of the acetylated derivatives of the compound and also derivatives formed by oxidation of the methyl group to a benzoic acid. As was commonly found with other materials, the toluenediamine was bound to nuclear DNA, to RNA and to the microsomal and soluble proteins of liver. Work with this compound is continuing in order to perfect chromatographic systems to separate metabolites.

Seemingly a very small proportion of the compound was converted to N-hydroxy compounds or precursors thereof. The synthetic study showed these compounds are very unstable and convert readily to azo or azoxy derivatives.

It was reported last year that despite the resistance of X/Gf mice to carcinogenesis by 2-acetylaminofluorene, they do excrete the important metabolic product N-hydroxy-N-2-fluorenylacetamide in amounts comparable to those excreted by sensitive mice. To help resolve further the question why one strain was more resistant than the other, comparative studies on the binding of 2-acetylaminofluorene to the cellular constituents were done. Levels were comparable in both strains. This leads to the conclusion that there must be some host factor (e.g. an immunological difference) available to the X/Gf mice which is not effective in the sensitive NIH Swiss mice.

During the past year a high pressure liquid chromatograph has been obtained and is now operating. This instrument is proving invaluable for the separation of metabolites of some of the high molecular weight amines which are being studied. One of these, 6-aminochrysene, was studied in depth during the past year or two. With the high pressure liquid chromatograph a very clear separation of 6-aminochrysene and some of its metabolites can be accomplished by this means. It is hoped to complete shortly the project on the metabolism of 6-aminochrysene.

During previous years a method was developed in this Branch for culturing rat liver parenchymal cells which can be transformed by various chemical carcinogens. Studies were then initiated on the biochemical characteristics of some of these cells, especially on aryl hydrocarbon hydroxylase (AHH) levels following repeated passages or after freezing. A comprehensive study showed that freezing did not affect AHH levels. An attempt, however, to determine whether AHH levels could be equated with the passage number of a cell line or with its transformation to a malignant type was inconclusive. Certain cell lines which had transformed, as evidenced by their production of tumors after injection in animals, had high AHH levels while another line had much lower levels. The AHH levels in several lines which had not transformed or were normal varied considerably. It appears, therefore, that enzyme levels cannot be used as a criteria for determination of whether a cell line has

transformed or is still normal. Another enzyme characteristic of liver cells, tyrosine *p*-aminotransferase is being studied to determine whether the results are different from or parallel those of the AHH study.

The Branch continues to study various factors which may influence chemical carcinogenesis. Thus, the effects of certain dietary constituents such as lipotropes, antioxidants and the like in inhibiting or enhancing the effects of the various chemical carcinogens are being continued. Various small scale bioassays to determine the effects of structure on the organotropic action of chemical carcinogens are being conducted, often in collaboration with various university groups. Colon tumor lines which were developed in this Branch are being continued and are available for collaborative studies with other research groups.

Analytical Chemistry Unit: Conducts independent research on the organic, analytical, and biological chemistry of nitrosamines and related compounds; and collaborates with other members of the Carcinogenesis staff in matters requiring chemical expertise, especially in problems of identifying non-polymeric organic molecules.

A well-equipped high resolution mass spectrometry laboratory provides the cornerstone of the Unit's intramural collaborative efforts, with services and collaborative assistance available to the research staff of the CMT Branch as well as of the entire Carcinogenesis Program. Structural analysis is feasible on nonpolymeric organic compounds in the solid, liquid or gaseous form. A gas chromatograph is interfaced directly with the mass spectrometer for analysis of those mixtures or their derivatives that can be fractionated by this type of chromatography. A variety of N-nitroso compounds, aromatic amines, polynuclear hydrocarbons, carbohydrates, alkyl halides and vinyl halides has been studied in collaboration with members of the Unit as well as with scientists outside the Unit. The mass spectrometry laboratory is involved in the following activities:

A number of mass spectra spectra were run on various synthetic 6-aminochrysene derivatives, with the aim of facilitating the structural elucidation of metabolic products from the parent compounds. Mass spectral measurements also assisted in the structure determination of synthetic intermediates produced during the preparation of various 2,4-diaminotoluene derivatives for metabolic studies. A publication describing the spectral characteristics of these compounds in in press.

In collaboration with members of the Chemistry Branch, mass spectrometric analysis is being used to determine the structure of benzo[*a*]pyrene metabolites in liquid chromatographic eluates.

Collaborative efforts with other scientists include development of an analytical method for structural determination of biologically important vitamin A conjugates (retinyl-phosphate-mannose type compounds) for the Lung Cancer Branch, and the analysis of tumor cell wall carbohydrates for the Viral Biology Branch, Viral Oncology Program.

In collaboration with the National Jewish Hospital and Research Center, Denver, Colorado, mass spectrometry is being used as the principal analytical tool in structural determination of the sulfolipids (di-, tri- and tetra-acyltrehalose derivatives) from *Mycobacterium tuberculosis*, Strain H37RV. A manuscript is in preparation. These chemical components may have some interesting immunological properties.

In collaboration with the Chemistry Department, University of Hawaii, Honolulu, gas chromatographic-mass spectrometric analysis provided essential characterization of chemical constituents, such as chlorine-, bromine- and iodine-containing haloforms, carbon tetrahalides, tetrahalopropenes, polyhalo-3-en-2-ones, monohaloacetones and 3,3-dihalo acrolein, in an edible seaweed *Asparagopsis taxiformis*. The presence of alkyl- and vinyl halides in foodstuffs is of concern here.

Some mass spectra are determined, on a time available basis, for investigators of the Drug Development Branch, Division of Cancer Treatment, NCI in return for use of other needed equipment, such as infrared and nuclear magnetic resonance instruments and a polarimeter.

The Unit's research activities are also aimed at understanding the fundamental organic chemical properties and reactivity of the carcinogenic N-nitroso compounds. Such data may provide important background leads for developing analytical methods necessary to determine the environmental distribution of these compounds, and clues as to the mechanism of their reactions as well as for understanding the biochemical and biological consequences of exposure to them.

Bacterial Metabolism Unit: Tests for mutagenesis or co-mutagenesis, using the Ames' technique, have shown that of 36 bile acids or their derivatives, only 5 were co-mutagenic when tested with 2-aminoanthracene activated with a rat liver homogenate. These included lithocholic, taurolithocholic, glycolithocholic, 3-keto-5-beta-cholanic, 3-beta-hydroxy-5-beta-cholanic acids and the ethyl and methyl esters of lithocholic acid. The co-mutagenic effect of the acid steroids was noted only with activated 2-aminoanthracene but not with such other carcinogens as 2-acetylaminofluorene, 3-methylcholanthrene, benz(a)pyrene, dimethylnitrosamine, or N-methyl-N'-nitro-N-nitrosoguanidine. None of the tryptophane metabolites tested have been mutagenic or co-mutagenic. Culture filtrates obtained from mixed fecal bacteria grown in tryptophane-containing medium were inactive by the Ames' test for mutagenesis.

The appearance of tryptophane metabolites was studied in the feces of germ-free rats maintained on a laboratory chow diet for one month and then for one month on a diet consisting only of autoclaved beef. Analyses showed that no metabolites were present in the feces of the animals on a meat diet while indole-3-acetic acid was found in samples from some animals on the chow diet. Several unidentified spots were observed on the TLC plates with samples from animals on either diet. Extraction methods were developed in this laboratory for the isolation and identification of tryptophane metabolites in feces. Fecal bacteria in tryptophane-containing medium have produced a number of tryptophane metabolites reported to induce bladder cancer. These include kynurenine, kynurenic acid, xanthurenic acid, and quinaldic acid as well as indole, skatole, indole-acetic and indole-pyruvic acids.

Project No. Z01-CP-04500-04-CMT
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mode of Action of Chemical Carcinogens -- The Mechanisms of the Appearance of α-Fetoproteins (AFP) in the Serum of Rats Given Chemical Carcinogens

Previous Serial Number: Same

Principal Investigator: James M. Sontag, Ph.D.

Other Investigators: Robert M. Kroes, D.V.M, Ph.D., Jerrold M. Ward, D.V.M., Ph.D., and Elizabeth K. Weisburger, Ph.D.

Cooperating Units: None

Man Years:

Total	:	0.2
Professional	:	0.1
Other	:	0.1

Project Description

Objectives:

A fetal globulin, α-fetoprotein (AFP) has been demonstrated to be present in the serum of some patients with hepatocellular carcinoma and a few other lesions such as teratomas. Depending on geographic location, the percentage of AFP-positive patients varies from 30% in some areas like the United States or Great Britain, to 80% in Africa. The presence of AFP is being developed as a diagnostic tool for hepatocellular carcinomas. A number of questions on the mechanism of formation and of the derepression of this fetal protein are of interest in relation to the etiology of hepatocellular carcinoma in man, such as age and the morphology of the tumor. Some of these basic questions were investigated in rats.

Methods Employed:

Utilizing amniotic fluid of inbred female Fischer strain rats at 16 to 20 days of gestation as antigen, antiserum was raised in rabbits. The antiserum was purified by specific techniques to secure an AFP-specific antiserum.

Double diffusion (Ouchterlony) techniques were utilized to assess the presence of AFP in serum of treated and control rats. In some cases confirmatory experiments were performed by electrophoresis and immunoelectrophoresis on cellulose acetate strips or on agarose coated slides.

In addition, radioimmune assays of selected serum samples have been performed by Dr. Sell, University of California, San Diego.

Major Findings:

Presence of α-fetoprotein (AFP) in serum of rats fed the hepatotoxin or hepatocarcinogen aflatoxin. Female Fischer rats were exposed to 2 ppm of aflatoxin B_1 from the time of conception to weaning of their offspring. The offspring received the same diets after weaning until their death. AFP levels have been followed in the sera of rats fed these diets. With the Ouchterlony technique it is practically impossible to detect AFP in the sera of rats fed low levels of aflatoxin B_1. However, with the radioimmune assay method of Dr. Sell, AFP levels in the serum samples were increased.

Significance to Biomedical Research and the Program of the Institute:

The current test series were inspired by the differences in the presence of a diagnostic antigen, α-fetoprotein (AFP) in the serum of human patients with liver cancer. The etiology of liver cancer in different parts of the world is unknown but presumably rests on a variety of inciting factors and chemical carcinogens. Model studies to pinpoint specific carcinogenic agents which would give rise to AFP and others which would not, might give clues to etiologic factors operating in man in various regions. At the same time an understanding of the factors controlling the release of a fetal antigen from normal or cancer cells might further our understanding of the mechanisms of carcinogenesis and thus eventually yield information which can be used to control and perhaps prevent cancer development. Studies on the effect of immunocompetence in the carcinogenic process likewise bear on these same problems and their resolution would certainly augment means of controlling the progress of the disease.

Proposed Course of Project:

Experimental work on this project has been terminated during the past year due to lack of personnel and the efforts of Dr. Borsos of the Biology Branch who is continuing such work under contract. The only activities during the past year were in preparation of a manuscript.

Publications

Kroes, R., Sontag, J. M., Sell, S., Williams, G. M., and Weisburger, J. H.: Elevated concentrations of serum α-fetoprotein in rats with chemically induced liver tumors. Cancer Res. (in press).

Kroes, R., Williams, G. M., and Weisburger, J. H.: On the precocious induction of α_1-fetoprotein by several hepatocarcinogens, including aflatoxin B_1, in rats. In Doll, R. and Vodopija, I. (Eds.): Host Environment Interactions in the Etiology of Cancer in Man. IARC Scientific Publicn. #7, FIC Proc. #18. International Agency for Research on Cancer, 1973, pp. 307-309.

Project No. Z01-CP-04580-02-CMT
1. Carcinogen Metabolism & Toxicology Branch, Carcinogenesis, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Role of Lipotropes in Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Lionel A. Poirier, Ph.D.

Other Investigators: Juanita Brown and Raymond Rusten

Cooperating Units: Dr. E.L.R. Stokstad, University of California, Berkeley
Dr. Russell Madison, Microbiological Associates
Dr. D.M. Matthews, Westminster Square Hospital, London
Dr. Adrianne Rogers, MIT, Cambridge, Massachusetts

Man Years:

Total	1.1
Professional:	0.3
Other:	0.8

Project Description

Objectives:

There is evidence that certain dietary deficiencies may lead to necrosis or fat deposition in the liver. Such morphological changes may be a prelude to the eventual development of a neoplastic condition. Therefore, investigations are being pursued on the extent to which the lipotropes choline and methionine inhibit, and vitamin B_{12} accelerates chemical carcinogenesis. The effects of these substances on chemical carcinogenesis by a variety of carcinogens are to be investigated. In addition, the mutual interactions of the lipotropes and the chemical carcinogens are to be determined.

Methods Employed:

The toxicity of 2-acetylaminofluorene (150 ppm in the diet), N,N-dimethyl-4-aminoazobenzene (700 ppm in the diet), aflatoxin (3 ppm in the diet), and diethylnitrosamine (40 ppm in the drinking water) and ethionine (3000 and 1000 ppm in diet) were studied in rats fed a vitamin B_{12}-deficient diet and the same diet supplemented with 100 µg/kg of vitamin B_{12}. S-Adenosylmethionine was determined using isotope-dilution techniques and thin-layer and column chromatography. Folate determinations were made using *L. casei*, *P. cerevisiae*,

and *S. fecalis*, following column chromatographic separations of liver folates. Vitamin B_{12} derivatives were studies by thin-layer chromatography and microbiological assays.

Major Findings:

1. Vitamin B_{12} increased the toxic activities of several carcinogens. The toxicities of 2-acetylaminofluorene, N,N-dimethyl-4-aminoazobenzene, diethylnitrosamine and aflatoxin toward rat liver was greater in vitamin B_{12}-adequate rats than in vitamin B_{12}-deficient rats; the increased toxicity in vitamin B_{12}-adequate rats could not be ascribed to differences in carcinogen intakes. In subacute toxicity studies ethionine was found to be more toxic to rats and mice fed a vitamin B_{12}-adequate diet than to animals fed a vitamin B_{12}-deficient diet. Ethionine toxicity was manifested by degeneration of the pancreas, liver and heart.

2. Diethylnitrosamine altered folate and B_{12} metabolism in rats. Collaborative studies done with Dr. E.L.R. Stokstad indicated that diethylnitrosamine administration to the livers of rats produced decreased hepatic levels of: folic acid, tetrahydrofolic acid, and the polyglutamates of folic acid, S-adenosylmethionine and CO_2 production from histidine. Diethylnitrosamine administration also increased the hepatic levels of N^5-methyltetrahydrofolic acid and FIGLU. All such changes have been reported in the livers of methyl-deficient rats. The decrease in hepatic S-adenosylmethionine caused by diethylnitrosamine could be reversed by methionine. Studies done with Dr. Matthews showed that methylcobalamine constituted an increased fraction of the total vitamin B_{12} content in a) the livers of rats treated with an acute or chronic dose of diethylnitrosamine, b) the Morris hepatomas 7777 and 7800 and c) the livers of tumor-bearing rats, when compared to the corresponding fraction of methylcobalamine in normal liver. Studies done with Dr. Rogers indicated that a lipotrope-deficient diet, which increased the carcinogenic activity of AAF towards the livers of male rats, did not increase the percent of N-hydroxy-AAF metabolites found in the urine. A new method has been developed in this laboratory for a more simple determination of S-adenosylmethionine in tissues.

Significance to Biomedical Research and the Program of the Institute:

The experiments under this project aim to delineate the exact roles of choline, methionine, vitamin B_{12} and folic acid in chemical carcinogenesis in the hope of eventually using these dietary factors to prevent or inhibit human cancer.

Proposed Course of Project:

The effect of vitamin B_{12}-deficiency on the carcinogenic activity of the following compounds will be investigated: ethionine, dimethylhydrazine, ethylnitrosourea, methylnitronitrosoguanidine, dimethylbenzanthracene, dimethylnitrosamine, and FANFT. This list of compounds will extend the target organs in which the role of vitamin B_{12} will be studied. The effect of vitamin B_{12} on the carcinogenic activity of a single carcinogen will be

investigated in detail. The effects of lipotropes on carcinogen metabolism and of carcinogens on lipotrope metabolism in target tissues will be determined. The results from these experiments, as well as those from collaborating laboratories should provide a critical appraisal of the role of lipotropes in chemical carcinogenesis.

Honors and Awards

Invited Lecturer: One Carbon Compounds in Carcinogenesis, Department of Nutritional Sciences, MIT, Cambridge, January 31, 1974.

Publications

Poirier, L. A.: Hepatocarcinogenesis by diethylnitrosamine in rats fed high dietary levels of lipotropes. J. Nat. Cancer Inst. (in press).

Poirier, L. A. and Rusten, R. T.: A new method for the rapid determination of S-adenosylmethionine in tissue. Anal. Biochem. (in press).

Project No. Z01-CP-04617-10-CMT
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Carcinogen Screening Operations

Previous Serial Number: Same

Principal Investigator: Elizabeth K. Weisburger, Ph.D.

Other Investigators: Lionel A. Poirier, Ph.D., James M. Sontag, Ph.D., Jerrold M. Ward, D.V.M., Ph.D., Timothy Benjamin, B.S., Carolyn A. Brown, M.S., and Juanita Brown

Cooperating Units: Dr. M. Shimkin, University of California, La Jolla
Dr. B. Ulland, FCRC
Dr. R. Madison, Microbiological Associates, Inc.
Dr. M. Wenk, Microbiological Associates, Inc.
Dr. M. Mason, Mason Research Institute

Man Years:

Total	:	2.7
Professional	:	2.5
Other	:	0.2

Project Description

Objectives:

Responsibilities are assumed on the design, performance and evaluation of studies in either contract or inhouse laboratories on the chronic effect and carcinogenicity of chemicals or of mixtures of chemicals. The aim of these investigations is to gather information on hazards involved in handling certain chemicals or drugs, and also on the mechanisms underlying joint effects in mixtures. Of particular interest are such mixtures which tend to give increased or decreased overall carcinogenic effect. Of concern also is the methodology of carcinogen screening, with emphasis on improvements in sensitivity, speed and economy. Short-term assays such as those based on mutagenesis are under development, taking into account the need to secure biochemical activation of most environmental agents. Guidelines for assessment of the carcinogenic potential of chemicals are developed, by consideration of their chemical structures, metabolic pathways and mutagenic activity.

Methods Employed:

Attempts are made to secure information on chemicals representing the greatest hazard to the largest number of people. Chemicals and drugs are rated as to priority on this basis as well as other criteria such as relationship to known carcinogens, epidemiologic observations, indications of specific toxicity and related factors. Standard protocols as well as newly designed protocols attempting to increase the sensitivity and speed of such tests are utilized. The carcinogenic and mutagenic activities of various compounds are determined in appropriate systems, either directly or after metabolic activation. Exploratory meetings with relevant national and international organizations in industry, in government and in university environments are setting the stage for a concerted developmental effort in this area.

Major Findings:

In the current year several experiments in contract laboratories have been completed and statistical evaluation is underway.

1. Industrial or environmental chemicals. Indications from a more rapid bioassay system using the strain A mouse are that some of the simple halogenated compounds may cause an increase in pulmonary adenoma. Reagents which are commonly used in the chemical and analytical laboratory for identification of materials by gas liquid chromatography have also increased the incidence of pulmonary adenomas in strain A mice.

2. Mutagenicity prescreens for chemical carcinogens. This is basically a contract program to determine the extent of correlation between chemical carcinogenesis and chemical mutagenesis. The original list of 85 compounds has been expanded to approximately 100 including procarcinogens, known carcinogens, ultimate carcinogens and mutagens which are on test for their activity. The spectrum of mutagenic activity includes DNA repair, gene mutations in mouse lymphoma cells, and microbial gene mutations. The latter are to be measured in yeat D3, five strains of histidine-requiring *Salmonella* and repair-deficient *E. coli*. All strains selected are to be tested *in vitro* with metabolic activation using rat liver post-mitochondrial supernatant for their sensitivity towards each of the 103 compounds. The current status of this effort may be described as follows: 1) Using a combination of microbial tester strains *in vitro* a positive response was obtained with 85% of the carcinogens tested and approximately 20% of the non-carcinogens. 2) The host-mediated bioassay using *Salmonella* or yeast as the tester strains were significantly less sensitive than the *in vitro* prescreens. The host-mediated assay, however, may still be useful with certain chemicals or specific classes of chemicals. 3) The mouse lymphoma system is not yet ready for the routine prescreening of large numbers of potential chemical carcinogens. 4) DNA repair in mammalian cells is still somewhat developmental, but has been successfully linked with microsomal drug activation *in vitro*.

3. Auramine carcinogenicity and metabolism. The major aim of this investigation is to determine whether the reactive moiety of auramine is the methyleneimine bridge between the two benzene rings or the dimethylamino group at either end of the molecule. Both have the potential to react as reactive electrophiles. Auramine is being tested as a direct-acting carcinogen by repeated skin painting in mice. Chemical analogues currently being tested in the same carcinogenicity test include: Michler's ketone, Michler's hydrol and *p*-rosaniline. These tests are currently in their 20th week of a 26-week experiment. Each of the compounds was painted on backs of mice twice a week at a level of 1 mg per painting for 10 weeks. None of the compounds exhibited appreciable toxicity. It is hoped that these experiments will provide some evidence as to the mode of action of auramine and structurally similar compounds.

4. Effect of phenobarbital on carcinogenic activity of diethylnitrosamine. A study by Peraino *et al.* showed that feeding of phenobarbital concurrently with 2-acetylaminofluorene inhibited liver carcinogenesis. However, if phenobarbital were fed after a period of 2-acetylaminofluorene feeding the incidence of liver tumors was increased. It was decided to study the effect with the liver carcinogen diethylnitrosamine (DEN). Additionally, it was decided to check the effects of hydroxyurea, an inhibitor of nucleic acid synthesis, and antilymphocytic serum immune suppression on the action of DEN. Concurrent feeding of phenobarbital with DEN decreased slightly the incidence of cancer of the liver and definitely decreased the incidence of lung metastases. However, when phenobarbital was fed beginning after a period of DEN intake liver cancer was increased significantly. Neither hydroxyurea nor antilymphocytic serum had any effect on the outcome on the enhancing or inhibiting processes.

5. Effect of strain on carcinogenicity of 1,4-bis-4-fluorophenyl-2-propynyl-N-cyclooctylcarbamate. It had been discovered by Dr. Paul Harris that several acetylenic cyclic carbamates which had been proposed as cancer chemotherapeutic agents were strong intestinal carcinogens in Harlan strain rats. In view of the interest in intestinal cancer it was decided to try the effects of one of these compounds in Charles River, Sprague-Dawley and Fischer strain derived rats. The compound was much more toxic in Fischer derived rats than in the Sprague-Dawley type. The interesting finding though was that in these two strains very few colon tumors were induced. The prominent tumors were carcinomas of the small intestine, squamous cell carcinoma of the ear duct, and lymphomas and leukemias. Of interest, there was a high incidence of mammary adenocarcinoma in the male Sprague-Dawley derived rats. A paper describing these results is now in press.

Significance to Biomedical Research and the Program of the Institute:

Many factors in the environment have not yet been evaluated for chronic toxicity and possible carcinogenicity. It is important to secure such information after deliberate and judicious setting of priorities. Cancer in man has been observed in the past as a result of exposure to chemicals

subsequently demonstrated to be carcinogenic. The aim of this program is to forestall such unintentional exposures of man which would result in neoplastic disease in the future.

Proposed Course of Project:

Continuing efforts are required 1) to assess relative priorities of materials to be tested, 2) to undertake studies on variations in the protocols to simplify or to develop novel test techniques which are more sensitive, more specific and more economic, and 3) to apply such tests to identify carcinogenic hazards in the environment.

Honors and Awards

Lionel A. Poirier:

Invited Lecturer: Electrophiles in Chemical Carcinogenesis. Battelle Laboratories, Columbus, Ohio, February 15, 1974

Invited Lecturer: Chemical Carcinogenesis Medical Oncology. National Center for Toxicological Research Advisory Board, Clinical Center, NIH, Bethesda, Maryland, November 5, 1974

Invited Speaker: Interrelationships between Diethylnitrosamine Hepatocarcinogenesis and Lipotrope Metabolism. XIth International Cancer Congress, Florence, Italy, October 24, 1974

Invited Speaker: Vitamin B_{12} Acceleration of Hepatocarcinogenesis. 65th Annual Meeting of the American Association for Cancer Research, Houston, Texas, March 27-30, 1974

Invited Speaker: National Cancer Advisory Board, October 7, 1974

Invited Speaker: HEW Committee on Environmental Toxicology, Bethesda, Maryland, January 21, 1974

Member, United States Interagency Panel on Environmental Mutagens

Member, Information and Resources Segment

Member, Carcinogen Metabolism and Toxicology Segment

Member, Chemical Selection Working Group

Co-organizer: U.S./Japan Workshop on Mutagenicity of Chemical Carcinogens, Honolulu, Hawaii, December 8-11, 1974

Elizabeth K. Weisburger:

Invited Speaker: Animal Models in Chemical Carcinogenesis. Gordon Research Conference on Drug Metabolism, Holderness School, Plymouth, New Hampshire, July 1974.

Invited Lecturer: Chemical Carcinogenesis. Department of Pathology Course for Graduate Students, University of Maryland, Baltimore, Maryland, August 12, 1974.

Invited Colloquium Speaker: Chemical Carcinogenesis. Department of Chemistry, Howard University, Washington, D. C., September 6, 1974.

Invited Lecturer: Identification of Environmental Carcinogens. Session on Environmental Carcinogenesis, Dayton, Ohio, September 24-26, 1974.

Invited Lecturer: Selection of Compounds for Carcinogenesis. Group of Center Grants in Toxicology, sponsored by National Institute of Environmental Health Sciences, Boston, Massachusetts, October 7, 1974.

Invited Lecturer: Chemical Carcinogenesis. Naval Ship Research and Development Center, Annapolis, Maryland, November 6, 1974.

Invited Participant: Working Group on the Evaluation of Carcinogenic Risk of Chemicals to Man. Lyon, France, November 26-December 3, 1974.

Invited Lecturer: Bioassay Protocols. Division of Toxicology, Food and Drug Administration, Washington, D. C., December 17, 1974.

Chairman, Session on Chemical Carcinogenesis, Annual Meeting of the Society of Toxicology, Williamsburg, Virginia, March 9-13, 1975.

Chairman, Session on Current Concepts of Carcinogenesis, Conference on Occupational Carcinogenesis, New York, New York, March 24-27, 1975.

Consultant, Special Consultative Panel on Hazards of Chemical Pesticides sponsored by the Environmental Studies Board, National Research Council, National Academy of Sciences

Participant, Subcommittee on NTA

Consultant, Naval Ship Research and Development Center

Consultant, National Science Foundation on Priority Ranking of Manufactured Organic Chemicals

Coordinator and Speaker for the Carcinogenesis Collaborative Conference in Orlando, Florida, February 1975

Chairman, Chemical Selection Working Group

Member, Bioassay Segment Advisory Group

Director, Carcinogen Metabolism and Toxicology Segment

Member, Carcinogenesis Contract Program Management Group

Consultant, Surgical Drugs Advisory Board, Food and Drug Administration, 1972-1974

Assistant Editor-in-Chief, *Journal of the National Cancer Institute*

Member, Editorial Advisory Committee of the American Association for Cancer Research for the journal, *Cancer Research*

Member, Editorial Board, *Excerpta Medica (Cancer)*

Trustee, Lebanon Valley College, 1970-1976

Publications

Baker, J. R., Mason, M., Yerganian, G., Weisburger, E. K., and Weisburger, J. H.: Induction of tumors of the stomach and esophagus in inbred Chinese hamsters by oral diethylnitrosamine. Proc. Soc. Exp. Biol. Med. 146: 291-293, 1974.

Baker, J. R., Smith, E. R., Yoon, Y. H., Wade, G. G., Rosenkrantz, H., Schmall, B., Weisburger, J. H., and Weisburger, E. K.: Nephrotoxic effect of 2-aminoanthraquinone in Fischer rats. J. Toxicol. Environ. Health 1: 1-11, 1975.

Homburger, F., Russfield, A. B., Weisburger, J. H., Lim, S., Chak, S., and Weisburger, E. K.: The pathology of aging CD® -1 HaM/IcR mice reared under standard laboratory conditions. J. Nat. Cancer Inst. (in press).

Poirier, L. A., Stoner, G. D., and Shimkin, M. B.: Bioassay of alkyl halides and nucleotide base analogs by pulmonary tumor response in strain A mice. Cancer Res. (in press).

Russfield, A. B., Homburger, F., Boger, E., Van Dongen, C. G., Weisburger, E. K., and Weisburger, J. H.: The carcinogenic effect of 4,4'-methylene-bis-(2-chloroaniline) in mice and rats. Toxicol. Appl. Pharmac. 31: 47-54, 1975.

Panel on Carcinogenesis - Falk, H. L., Diamond, L., Friedman, L., Sydnor, K. L., and Weisburger, E. K.: Chemical carcinogenesis. In Principles for Evaluationg Chemicals in the Environment, National Academy of Sciences, Washington, D. C., 1975, pp. 134-155.

Stoltz, D. R., Poirier, L. A., Irving, C. C., Stich, H. F., Weisburger, J. H. and Grice, H. C.: Evaluation of short-term tests for carcinogenicity. Toxicol. Appl. Pharmac. 29: 157-180, 1974.

Stoner, G. D., Weisburger, E. K., and Shimkin, M. B.: Tumor response in strain A mice exposed to silylating compounds employed for gas-liquid chromatography. J. Nat. Cancer Inst. (in press).

Ward, J. M. and Habermann, R. T.: Pathology of stomach cancer induced by agricultural chemicals ethylene dibromide and dibromochloropropane. Bull. Soc. Pharm. Environ. Path. 2: 10-11, 1974.

Weisburger, E. K., Ulland, B. M., Schueler, R. L., Weisburger, J. H., and Harris, J. H.: Carcinogenicity of 3 dose levels of 1,4-bis(4-fluorophenyl) 2-propynyl-N-cyclooctylcarbamate in male Charles River and Fischer strain rats. J. Nat. Cancer Inst. (in press).

Weisburger, J. H., Griswold, D. P., Jr., Prejean, J. D., Casey, A. E., Wood, H. B., Jr., and Weisburger, E. K.: The carcinogenic properties of some of the principal drugs used in clinical cancer chemotherapy. Recent Results in Cancer Research (in press).

Weisburger, J. H., Ward, J. M., Madison, R. M., Viguera, C., and Weisburger, E. K.: Modification of diethylnitrosamine liver carcinogenesis with phenobarbital but not with immunosuppression. J. Nat. Cancer Inst. (in press).

Project No. Z01-CP-04618-10-CMT
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mode of Action of Chemical Carcinogens -- Endogenous and Exogenous Factors in Chemical Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Elizabeth K. Weisburger, Ph.D.

Other Investigators: Lionel A. Poirier, Ph.D., Jerrold M. Ward, D.V.M.,Ph.D., Ritva P. Evarts, D.V.M.,Ph.D., Timothy Benjamin, B.S., Carolyn A. Brown, M.S., and Thirayudh Glinsukon, Ph.D.

Cooperating Units: Dr. John Baker, University of Mississippi
Dr. Emil H. White, Johns Hopkins University
Dr. C. Chen, Northwestern University

Man Years:

Total	:	4.3
Professional	:	2.3
Other	:	2.0

Project Description

Objectives:

Investigations to discover the intimate factors involved in the initiation and development of neoplasia are explored by means of animal experiments in various species. The studies utilized a number of chemical carcinogens administered to animals under varying conditions of diet, endocrine situation or immunological status so as to gain insight into controlling mechanisms in the initiation and development of chemically induced tumors. Further efforts deal with the development of systems which lead to the prevention of the processes of chemical carcinogenesis and a study of the mechanisms related thereto.

Methods Employed:

Chemical carcinogens or unknown agents to be evaluated for carcinogenicity are administered by a number of routes to several strains of rats or mice on specific dietary regimens. Additionally, certain chemicals with specifically tailored structures or properties are given together with chemical carcinogens

to determine whether tumor induction can be delayed or inhibited. Host factors such as the endocrine or immunologic status are being investigated in relation to the effectiveness of tumor formation. After judiciously selected treatment and observation periods, autopsies of the experimental animals are performed. Upon histologic processing of tissues, the results in experimental systems are carefully evaluated in relation to control groups.

Major Findings:

1. Effects of benzene in newborn mice. There are many case reports ascribing leukemia or disorders of the hematopoietic system in man to benzene but no experimental animal model seems to simulate this effect. In a study in newborn C57Bl mice which are relatively sensitive to many carcinogens, it was found that subcutaneous injection of benzene in corn oil was not effective. Amyloid disease was the only deleterious action of the benzene treatment.

2. Effect of aflatoxin on fetus. Aflatoxin is suspected as a cause of liver cancer in some parts of the world where high humidity and poor storage conditions lead to rapid contamination of the peanut or other grain crops. In order to simulate these conditions, female rats received 2 ppm of aflatoxin B_1 from the time of conception to weaning of the offspring. The offspring received the same diet after weaning until death while another group of Fischer rats received 2 ppm of aflatoxin from 6 weeks of age until death. Early exposure to aflatoxin resulted in a decrease in survival time and more of the rats exposed *in utero* died from anaplastic liver tumors and hemorrhage than did rats exposed at 6 weeks of age onward. A publication describing these results is in press.

3. Induction of intestinal tumors in mice with butylnitrosourea. Administration of butylnitrosourea (BNU) in the drinking water to C57Bl mice led to a high incidence of thymic lymphomas and a few intestinal tumors. However, one injection of BNU led to many intestinal tumors but few leukemias.

4. Effects of structure on chronic toxicity of nitrosamides. In cooperation with Dr. Emil White of Johns Hopkins, a series of related nitrosamides is being tested in strain A mice.

5. Chronic effects of benzoxazoles from grains. In cooperation with Dr. John Baker, University of Mississippi, the effects of various benzoxazoles isolated from grains, plus possible nitroso derivatives of these, are being studied in NIH Swiss mice.

6. Peroxide of 7,12-dimethylbenz(a)anthracene (DMBA). The comparative effects of DMBA and DMBA peroxide are studied in strain A mice. The peroxide was much less toxic than DMBA itself.

7. Toxicity studies on acetyl derivatives of 2,4-toluenediamine. As part of our coordinated study on this industrial intermediate, toxicity studies on the various acetylated derivatives are being followed. The free amine was more toxic than the acetylated derivatives, indicating that acetylation is probably a major detoxification route for 2,4-toluenediamine.

8. Inhibitory effect of hydroxylamine on spontaneous mammary tumors of C3H mice. Several years ago it was found that hydroxylamine inhibited the development of mammary tumors in female C3H mice. A sequential study of this effect of hydroxylamine has begun to determine whether the physical development of the mammary gland is inhibited and whether pituitary prolactin levels may be involved. Whole mounts of mammary glands are being made for comparison along with electrophoretic studies for prolactin determination.

Significance to Biomedical Research and the Program of the Institute:

The prevention and cure of neoplasia hinges on an understanding of the intimate factors involved in the pathogenesis of cancer. The research performed within the framework of this report aims to produce data which will permit the comprehension of the underlying mechanisms involved in chemical carcinogenesis.

Proposed Course of Project:

A number of the tests for chronic toxicity and carcinogenicity presently underway or planned to go on stream shortly will require considerable effort for several years. Additional emphasis will be placed on methods for inhibiting the carcinogenic process and on understanding the various steps leading to such inhibitions. Thus studies will include variations in endogenous factors as well as select exogenous materials.

Publications

Cohen, A. E., Weisburger, E. K., Weisburger, J. H., Ward, J. M., and Putnam, C. L.: Cystoscopy of chemically-induced bladder neoplasms in rabbits administered the carcinogen dibutylnitrosamine. Investig. Urol. 12: 262-266, 1975.

Ward, J. M., Sontag, J. M., Weisburger, E. K., and Brown, C. A.: Effect of lifetime exposure to aflatoxin B_1 in rats. J. Natl. Cancer Inst. (in press).

Ward, J. M. and Weisburger, E. K.: Induction of intestinal tumors in mice by a single injection of N-nitroso-N-butylurea. Cancer Res. (in press).

Ward, J. M., Weisburger, E. K., Benjamin, T., and Moss, R. A.: Shift of organotropy of azoxy compounds - Replacement of methyl with an octyl group. J. Natl. Cancer Inst. 53: 1181-1185, 1974.

Ward, J. M., Weisburger, J. H., Yamamoto, R. S., Benjamin, T., Brown, C. A., and Weisburger, E. K.: Long-term effect of benzene in C57BL/6N mice. Arch. Environ. Health 30: 22-25, 1975.

Weisburger, E. K.: A critical evaluation of the methods used for determining carcinogenicity. J. Clin. Pharmac. New Drugs 15: 5-15, 1975.

Weisburger, E. K.: Identification of environmental carcinogens. Proc. 5th Conf. on Environmental Toxicology (in press).

Weisburger, E. K., Ward, J. M., and Brown, C. A.: Dibenamine: Selective protection against diethylnitrosamine-induced hepatic carcinogenesis but not oral, pharyngeal and esophageal carcinogenesis. Toxicol. Appl. Pharmac. 28: 477-484, 1974.

Project No. Z01-CP-04680-06-CMT
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mode of Action of Chemical Carcinogens -- Development and Application of *In Vitro* Systems Involving Epithelial Cells

Previous Serial Number: Same

Principal Investigators: Jane Idoine, B.S. and Elizabeth K. Weisburger, Ph.D.

Other Investigators: Jerry Elliott, B.S. and Mary Wilson, M.S.

Cooperating Units: Dr. J. Rhim, Microbiological Associates
Dr. L. Keefer, Analytical Chemistry Unit, CMT, NCI
Dr. P. Roller, Analytical Chemistry Unit, CMT, NCI

Man Years:

Total	:	1.6
Professional	:	0.3
Other	:	1.3

Project Description

Objectives:

Successful culture of epithelial-like cells derived from livers of 8 to 10 day old Fischer strain 344 rats and malignant transformation of such cultured cells by a variety of chemical carcinogens were discussed in previous reports. Since neoplasia in man and animals involves mainly epithelial cells, our principal aim in current investigations has been to further determine the potential of *in vitro* epithelial systems for use in rapid bioassay procedures for chemical carcinogens and in examining the biochemical mechanisms of carcinogenesis.

Two problems encountered in past investigations were the relatively long time required to develop cultures that were proliferating adequately to provide enough cells for biochemical investigations and the fact that some sublines of control cells underwent spontaneous malignant transformation as demonstrated by tumor formation following injection of cells into syngeneic hosts. Unfortunately, no distinctive morphological changes were apparent in the cultured cells that induced tumors. In addition, the spontaneous

transformation was not correlated with a specific time in culture nor with the number of times a line had undergone subculturing. Therefore, we have emphasized the development of methods for producing a greater number of cultures with similar characteristics so that studies can be repeated as required in more replicable systems. Investigation of pertinent biochemical properties of untreated or chemically exposed cultured cells in respect to the effects of various cultural conditions and in comparison to the characteristics of liver tissue from whole animals should help assess the value of this *in vitro* system.

Methods Employed:

Cell culture methods designed for selection of epithelial cells were employed to initiate cell cultures from livers of 8 to 10 day old Fischer rats. The method for selection of epithelial cells developed in this laboratory was modified by detaching and transferring islands of epithelial cells so that many homogenous sublines could be cultured from one primary culture. Frozen stocks of all sublines were prepared as soon as cultures were proliferating adequately, and some sublines were cultured continuously under conditions where continuing growth and proliferation were maintained. Transformation was monitored by testing cells for growth in soft agar (Sachs' method) and for ability to induce tumors after injection into animals. Biochemical procedures were applied to characterize these cells. Base levels and inducible levels of aryl hydrocarbon hydroxylase (AHH) activity were determined in homogenates of whole cells to evaluate the functional quality of the cultured cells under various conditions. Chromosome preparations of cell lines were done periodically and karyotyping done.

Major Findings:

1. Effect of various conditions of culture on hepatic cells *in vitro*. Four sublines were maintained in continuous culture for 7 to 9 months and tested at intervals for levels of AHH activity, ability to grow in soft agar, and to induce tumors after inoculation into animals. At passages 20 to 22, 3 of the 4 sublines under study demonstrated only 15 to 35% of the AHH activity observed in cells of early passages (P4 to P8), as described, while 75% of the initial activity was observed in cells of passage 22 of the fourth subline. Morphologically, all four lines appear primarily epithelial in nature as they did when first tested, although in one of the lines with sharply decreased AHH activity some spindle cells are now observed. Irradiated weanling Fischer rats inoculated with cells from the four sublines at intervals up to passage 43 (approximately 9 months in culture) have not developed tumors. Animals are routinely sacrificed one year after inoculation. The four sublines do not grow in soft agar.

2. Effect of frozen storage on cultured epithelial cells. Frozen stocks of cells prepared from cultures at the earliest possible passage levels could provide the means for experimentation in more comparable cultures than those available from lines in continuous culture. To assess the quality of cultures

prepared from frozen stocks, a continuing study is underway to determine the effects of frozen storage on the biochemical characteristics of cultured epithelial cells and on their tendency toward spontaneous transformation. Portions of a pooled suspension of cells from one of the sublines described were frozen in a number of separate vials. Other parallel cultures of the same subline were used for control testing of the condition of the cells not subjected to freezing. At intervals of 24 hours, 1 month, 4 months, 8 months, and 12 months, some of the frozen cells were thawed, cultured and subcultured two or three times to obtain enough cells for AHH assays, soft agar tests, and animal inoculations. The results from all of the various thawings indicate that these cells have the same high AHH activity observed in the cultures at the passage level from which the frozen stocks were prepared, while cells of the same line carried in continuous culture over this same period had decreased AHH activity. The thawed and cultured cells also do not grow in soft agar. Animals have been sacrificed one year after implantation for the 24 hours and one month intervals and no tumors have been observed. Tumors as detected by palpation have not been noted in the other animals injected with cells from the later thawing intervals.

3. Inducibility of AHH activity in cultured epithelial cells. Some of the sublines of epithelial cells that had been cultured continuously were tested for inducibility of AHH activity by benz(a)anthracene, 3-methylcholanthrene and 7,12-dimethylbenz(a)anthracene. All three chemicals induced significantly higher levels of AHH activity in the cultured cells.

4. Caffeine as a promoter or inhibitor of chemical transformation. There have been conflicting opinions as to whether caffeine is a promoter or inhibitor of cell transformation when the cells are exposed to a known carcinogen along with the caffeine. However, as yet no one has investigated this in liver cells.

Normal epithelial cells were exposed to caffeine, at various concentrations, with and without a known carcinogen, aflatoxin B_1. This was done to determine toxicity levels as well as the ability to promote or inhibit transformation. Unfortunately the levels of caffeine used did not exhibit toxicity. After the various exposures the resulting sublines were checked for their ability to grow in soft agar but the cells still would not grow in soft agar. Cells were also injected into irradiated weanlings at intervals of one month and two months after exposure. As yet none of the animals have developed tumors from the injection. These animals will continue to be monitored for one year after injection and then sacrificed.

Similar experiments with other carcinogens are planned.

5. Toxicity and transforming action of dimethylnitrosamine (DMN) and derivatives. Three compounds, dimethylnitrosamine (DMN) and its acetoxy (DMNOAc) and methoxy (DMNOMe) derivatives provided by Drs. Keefer and Roller were tested to determine their ability to transform liver cells. In preliminary

toxicity studies, the DMNOAc was the most toxic of the three. After exposure to the compounds the cells were checked for growth in soft agar and their ability to produce tumors after inoculation but the results are negative thus far.

Significance to Biomedical Research and the Program of the Institute:

An assay system which can be designed to give results more rapidly, specifically, and reliably than the customary long-term tests for carcinogenicity is highly desirable with the dual purpose of studying fundamental phenomena and the detection of harmful agents in our environment. Full exploitation of workable *in vitro* systems has not been achieved. It is our goal to develop such systems; an additional advantage of the *in vitro* systems is that eventually they can be established utilizing cells from man. Hence carcinogenesis *in vitro* with human cells avoids the question of species difference, so often raised in evaluating the significance of carcinogen bioassay systems.

Proposed Course of Project:

These epithelial cells will be carefully examined for suitability as a carcinogen screening system. The relevant morphological properties in both normal and carcinogen treated cells will be investigated thoroughly. Extensive efforts will deal with the biochemical properties of both the normal and the transformed cells in culture. Basic biochemical mechanisms of the system will be investigated.

Honors and Awards

Elizabeth K. Weisburger:

Invited Discussant: Workshop on Rat Liver Pathology, Cape Cod, September 30-October 4, 1974.

Project No. Z01-CP-04688-05-CMT
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Office of the Chief
3. Frederick, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mode of Action of Chemical Carcinogens -- Investigations of Systems Leading to Cancer in the Colon and Small Intestine

Previous Serial Number: Same

Principal Investigator: Elizabeth K. Weisburger, Ph.D.

Other Investigators: Carolyn A. Brown, M.S., Jerrold M. Ward, D.V.M.,Ph.D., Timothy Benjamin, B.S., Ritva Evarts, D.V.M.,Ph.D., and Robert C. Bast, Jr., M.D.

Cooperating Units: Biology Branch, NCI

Man Years:

Total	2.0
Professional	0.5
Other	1.5

Project Description

Objectives:

In the United States and Western Europe the mortality rate due to colon and rectum cancer in males is second only to lung cancer, and in females second to breast cancer. In incidence, colon cancer is third after skin and lung in men, and in women, after breast and uterus. However, in other countries such as Japan and Africa, colon cancer incidence is much lower, thus suggesting that environmental factors are associated with the etiology of this important disease. Within recent years several new agents were discovered by which colon cancer could be induced in animals. The goal of the studies to be described is to evaluate how select environmental factors affect the production of cancer of the colon in animals thereby gaining insight into conditions possibly affecting humans.

Methods Employed:

Rats, mice and other rodent species were treated with chemicals known to lead to colon cancer. These include 1,2-dimethylhydrazine and azoxymethane. The animals are placed on diets mimicking those utilized in various parts of the world with regard to quantity and quality of protein, fat, carbohydrates, micronutrients, and the amount of bulk material such as cellulose in the diet. After appropriate periods of time the animals are carefully autopsied and the lesions are examined microscopically.

Major Findings:

1. Attempt to inhibit growth of transplanted colon tumors by BCG. In cooperation with Dr. Robert Bast, Biology Branch, trials with BCG (Ribi vaccine) were done in rats implanted with the transplantable colon tumors developed in this Branch. A number of technical problems were encountered, including changes in the antigenicities of the tumors. The vaccine treatment did not appear to inhibit the tumor growth.

2. Transplantation of chemically induced jejunal and colonic tumors in rats. Jejunal and colonic mucinous adenocarcinomas developed in rats injected with azoxymethane. To determine whether the tumors remain viable when frozen, samples were frozen for 4 months. The tumors were then thawed and injected into weanling rats to determine their viability and histologic similarity to the original tumors.

3. β-Glucuronidase and cytochrome oxidase in colon neoplasms from rats injected with azoxymethane. Rats are injected once weekly for 10 weeks with a dose of azoxymethane which leads to many tumors, well distributed throughout the colon. Rats are killed at 4, 6 and 8 months after the first injection. Tumors found at each period are being studied histologically and histochemically to determine whether there is any correlation between enzyme activity and the tumor type.

Significance to Biomedical Research and the Program of the Institute:

Establishment of a colon cancer model with similarities to the human disease was necessary to study the effects of diet on colon cancer induction. Dietary factors are thought to be responsible for the occurrence of human colon cancer. Our studies suggest that under our experimental conditions, diets containing nutrients thought to be important for the high incidence of human colon cancer in some parts of the world did not increase the incidence of chemically-induced cancer. The limitations of this model are obvious.

Proposed Course of Project:

To complete all the experimental studies noted above in order to a) characterize the pathology of the experimental model, b) determine the effects of various diets on colon cancer incidence in the experimental model, and c) establish and study the transplantable carcinomas noted above.

Publications

Ward, J. M.: Morphogenesis of chemically-induced neoplasms of the colon and small intestine in rats. Lab. Investig. 30: 505-513, 1974.

Ward, J. M.: Naturally occurring Sendai virus disease of mice. Lab. Animal Sci. 24: 938-942, 1974.

Ward, J. M., Yamamoto, R. S., Benjamin, T., Brown, C. A., and Weisburger, J. H.: Experimentally induced cancer of the colon in rats and mice. J. Amer. Vet. Med. Assoc. 164: 729-732, 1974.

Project No. Z01-CP-04542-03-CMT
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Analytical Chemistry Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Chemistry of N-Nitroso Compounds

Previous Serial Number: Same

Principal Investigator: Larry K. Keefer, Ph.D.

Other Investigators: Peter P. Roller, Ph.D., Elizabeth K. Weisburger, Ph.D., Jerry Rice, Ph.D., Sewa Rama Joshi, D.V.M., Ph.D., William Gaffield, Ph.D. (USDA), Walter Zielinski, Jr., Ph.D., Yuki Oshiro, Ph.D. (Frederick Cancer Research Center), William Lijinsky, Ph.D. (Biology Division, Oak Ridge National Laboratories), Winifred Palmer, Ph.D. (Frederick Cancer Research Center),Christopher Michejda, Ph.D. (National Science Foundation)

Cooperating Units: Metabolic Studies Section, CMT, NCI; Perinatal Carcinogenesis Section, EXP, NCI; Normal Volunteer Program; Western Regional Research Laboratory, USDA

Man Years:

Total	0.6
Professional	0.5
Other	0.1

Project Description

Objectives:

(1) To investigate mechanisms by which metal ions might promote the formation of N-nitroso compounds in the environment. (2) To study mechanisms of metabolism of and carcinogenesis by N-nitroso compounds using the deuterium isotope effect. (3) To study the reaction of N-nitroso compounds with protonating agents and other electrophilic species, making inferences where possible regarding the significance to carcinogenesis research of the reversible and irreversible chemical changes that ensue. (4) To synthesize possible metabolites of certain N-nitroso compounds for comparisons of their physical, chemical, and biological properties with those of the parent procarcinogens. (5) To synthesize a spin-labelled nitrosamine and characterize its precovalent interactions with cellular macromolecules by esr techniques.

(6) To elucidate mass spectrometric fragmentation mechanisms for N-nitroso compounds, especially by study of selectively deuterated substrates. (7) To study the circular dichroism of optically active nitrosamines with the goal of clarifying the sector rule for this series of compounds.

Methods Employed:

In addition to the standard methods of synthetic and mechanistic chemistry, considerable use has been made of the deuterium isotope effect as a pharmacological probe. This method has not been widely exploited elsewhere, and it is possible that this project may in the long run demonstrate the technique's general utility in pharmacological investigations.

Major Findings:

1. An efficient three-step synthesis has been devised for n-butyl-(3-carboxypropyl)nitrosamine (BCPN), a metabolite of dibutylnitrosamine, and itself a known bladder carcinogen in rats. Thus, this compound is now readily available for biological evaluation by cancer researchers. Cooperative work with Dr. Winifred Palmer of FCRC is in progress to use BCPN as an affinity chromatography substrate for the possible isolation of nitrosamine metabolizing enzymes from rat liver.

2. The gas chromatography-mass spectrometry system has been put to very valuable use in supporting synthetic and mechanistic studies on dialkylnitrosamines. Synthesis of several deuterium labeled dialkylnitrosamines is planned to elucidate molecular fragmentation processes taking place in the mass spectrometer.

3. Circular dichroism (CD) studies have established a sector rule useful for the structural determination of optically active nitrosamines. This rule is particularly helpful in determining the configuration of the nitroso group (syn or anti), and may be useful in studying biologically important α-oxygenated nitrosamines.

4. Several derivatives of DMN-OH, the hypothetical metabolic activation product of dimethylnitrosamine, have been prepared and characterized. Of the numerous chemical and biological reactivity studies we have conducted with this series of compounds, the most significant thus far have involved methyl-(acetoxymethyl) nitrosamine (DMN-OAc). Hydrolysis of this material in simple chemical media has provided the first experimental glimpses of DMN-OH, the prototype of a virtually unexplored family of compounds (the α-hydroxy nitrosamines) of possible crucial importance in carcinogenesis research. For example, after generation from DMN-OAc in the presence of hog liver esterase at neutral pH, the E conformer of DMN-OH has been shown to undergo very rapid decomposition (halflife of no more than a few minutes, possibly much less). Further, DMN-OH has been found to be significantly less selective as a methylating agent than dimethyl sulfate. Finally, biological tests done in collaboration with Dr. Jerry Rice et al. have shown that DMN-OAc is an intestinal carcinogen by i.p. injection in rats.

5. Studies of the deuterium isotope effect as a pharmacological tool have continued. Nitrosomorpholine-d_4 has been shown in collaboration with Dr. William Lijinsky to be significantly less potently hepatocarcinogenic than its undeuterated parent, consistent with the results of an earlier test of dimethylnitrosamine (DMN) and its hexadeuterated derivative (DMN-d_6). Dr. Yuki Oshiro's biochemical comparisons of DMN and DMN-d_6 have suggested implications of potential importance to the understanding of carcinogenesis mechanisms, such as multiple pathways for metabolism of DMN, and a possible mechanism for repair of methylated bases in damaged nucleic acids by active demethylation.

Significance to Biomedical Research and the Program of the Institute:

With considerable public attention focused on the possibility that N-nitroso compounds might be responsible for some human cancer, collection of data on the chemical, physical, and biological properties of this hitherto poorly understood class of compounds is an urgent matter. Certain aspects of the studies reported here may aid in developing methods for predicting and analyzing the environmental distribution of these potential carcinogens, while other aspects are aimed at shedding light on the biochemical mechanisms by which these compounds exhibit their untoward effects.

Proposed Course of Project:

The studies described above will be completed and published, and the important hypotheses and implications they suggest will be investigated as appropriate. In addition, new studies will be initiated. One program planned for the near future, for example, is the attempted synthesis of (Z) methyl(acetoxymethyl)nitrosamine for comparison of its solvolysis behavior with that of the (E) conformer mentioned in (5) above.

Honors and Awards

Member, Carcinogen Metabolism and Toxicology Segment, DCCP, NCI

Member, Information and Resources Segment, DCCP, NCI

Publications

Roller, P. P. and Keefer, L. K.: Catalysis of nitrosation reactions by electrophilic species. Proceedings of the Third Meeting on the Analysis and Formation of N-Nitroso Compounds, Lyon, France (in press).

Olah, G. A., Donovan, D. J. and Keefer, L. K.: Carcinogen chemistry I. Reactions of protonated dialkylnitrosamines leading to alkylating and aminoalkylating agents of potential metabolic significance. J. Natl. Cancer Inst. 54: 465-472, 1975.

Selkirk, J. K., Croy, R. G., Roller, P. P. and Gelboin, H. V.: High pressure liquid chromatographic analysis of benzo(a)pyrene metabolism and covalent binding and the mechanism of action of 7,8-benzoflavone and 1,2-epoxy-3,3,3 trichloropropane. Cancer Res. 34: 3474-3480, 1974.

Orme, T. W., Boone, C. W. and Roller, P. P.: The analysis of 2-acetamido-2-deoxyaldose derivatives by gas-liquid chromatography and mass spectrometry. Carbohydr. Res. 37: 261-266, 1974.

Burresone, B. J., Moore, R. E. and Roller, P. P.: Haloforms in the essential oil of the alga Asparagopsis taxiformis (rhodophyta). Tetrahedron Letters (in press).

Glinsukon, T., Weisburger, E. K. Benjamin, T. and Roller, P. P.: Preparation and spectra of some acyl derivatives of 2,4-diaminotoluene. J. Chem. Engi. Data (in press).

Project No. Z01-CP-04547-03-CMT
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Bacterial Metabolism Unit
3. Frederick, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Laboratory Study of the Etiology of Colonic Cancer

Previous Serial Number: Same

Principal Investigator: Sidney J. Silverman, Ph.D.

Other Investigators: Milton W. Slein, Ph.D., Mary J. Wilson, M.S., and A. W. Andrews (Litton Bionetics, Inc.)

Cooperating Units: Project 7, FCRC (Litton Bionetics, Inc.)

Man Years:

Total	3.2
Professional	1.2
Other	2.0

Project Description

Objectives:

(1) Study the metabolism and physiology of intestinal bacteria in relation to the production, activation, or inactivation of carcinogens. (2) Develop *in vitro* assays for mutagenesis for use in screening tests for carcinogens.

Methods Employed:

The degradation of acid steroids by several species of anaerobic intestinal bacteria is studied in pure or mixed cultures. The products of bacterial metabolism are identified by chromatographic and chemical procedures and their mutagenic activity is determined by *in vitro* methods.

Major Findings:

Of numerous acid steroids tested, only a few were found to be co-mutagenic when tested by the Ames' technique. When tested in conjunction with 2-amino-anthracene activated by liver microsomes, lithocholic, taurolithocholic, glycolithocholic, 3-keto-5β-cholanic and 3-beta-hydroxy-5-beta-cholanic acids, and the ethyl and methyl esters of lithocholic acid all significantly

increased the number of *Salmonella* revertants to histidine prototrophy. Cholesterol and a number of neutral sterols as well as tryptophane and its metabolites (reported as etiological agents of bladder cancer) failed to act as either mutagens or co-mutagens.

Co-mutagenicity was noted only with metabolically-activated 2-aminoanthracene. No adjuvant effect was observed when lithocholic acid and the related 7-dehydroxylated sterols were tested with 2-acetylaminofluorene, 3-methylcholanthrene, dimethylnitrosamine, or N-methyl-N'-nitro-N-nitrosoguanidine.

A simpler procedure was devised for the extraction of fecal steroids. The procedure recovered over 80% of the steroids and avoided the formation of artifacts.

Significance to Biomedical Research and the Program of the Institute:

Epidemiological evidence suggests that diet is an important factor in the causation of large bowel cancer. Consumption of beef was shown to be highly correlated with the incidence of the disease. These studies are an attempt to determine whether components of beef (tryptophane and its degradation products) or endogenous substances such as the acid and neutral sterols of the bile play a role in the etiology of colonic cancer. Proof of this cause-effect relationship and the metabolic processes that lead to the formation of carcinogens should aid in the prevention of large bowel cancer.

Proposed Course of Project:

Methods will be developed to provide more quantitative data with the Ames' mutagenesis test. Studies will be performed with suspensions of mixed fecal bacteria to investigate the synthesis, activation, and inactivation of intestinal carcinogens.

Honors and Awards

Session Convenor, Third International Symposium on Intestinal Microecology

Observer-Member, Colon-Rectum Cancer Advisory Committee to the National Large Bowel Cancer Project

Member, Medical Sciences Curriculum Committee, Frederick Community College

Project No. ZO1-CP-04619-10-CMT
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Metabolic Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mode of Action of Chemical Carcinogens -- Studies on the Metabolism of Chemical Carcinogens

Previous Serial Number: Same

Principal Investigator: Preston H. Grantham, M.S.

Other Investigators: Letitia C. Mohan, B.A., Thirayudh Glinsukon, Sc.D., Sunanda Gothoskar, Ph.D., Timothy Benjamin, B.S., Nathan Lewis, B.S., and Elizabeth K. Weisburger, Ph.D.

Cooperating Units: Analytical Chemistry Unit, CMT, NCI

Man Years:

Total	: 4.0
Professional	: 2.0
Other	: 2.0

Project Description

Objectives:

The aim of this research is to gather data relevant to the etiology of neoplasia at the molecular level. To this end chemical carcinogens, especially those of the aromatic amine type, are being utilized. Their metabolism and interaction with host tissues and specific targets are studied. Simplified yet realistic model systems are devised in order to gain an understanding of the fundamental events operating in chemical carcinogenesis. Specific inhibitors and accelerators of the carcinogenic process are applied as tools to develop information on and permit discrimination between biochemical events directly involved in the neoplastic change and those representing other reactions.

Methods Employed:

Biochemical and pharmacological techniques are applied to determine the metabolism of N-hydroxy-N-2-fluorenylacetamide and related compounds in various animal species. This includes isolation and purification of enzyme systems concerned with certain metabolic steps. Procedures are developed and applied for the separation, purification and analysis of macromolecular

constituents such as DNA, RNA and proteins from tissues of animals treated with chemical carcinogens and from control animals. The metabolism of chemical carcinogens and related compounds, and interaction with host targets are examined *in vivo* and *in vitro* in the presence of various chemicals or antibiotics which affect the carcinogenic process.

Major Findings:

1. Effect of *p*-hydroxyacetanilide feeding on body weights. As part of a continuing study on the inhibitory effect of *p*-hydroxyacetanilide (*p*-OH-AA) on the carcinogenicity of 2-fluorenylacetamide (FAA), the food intake and body weights of rats on FAA, *p*-OH-AA and FAA + *p*-OH-AA was investigated. The results showed that rats on the diet containing FAA + *p*-OH-AA actually consumed more of the carcinogen than did those on FAA alone.

2. Metabolism of 2-acetylaminofluorene in resistant and susceptible mice. In the report last year it was mentioned that the X/GF mice which are resistant to the carcinogenic action of 2-acetylaminofluorene (2-AAF) excrete the N-hydroxylated derivative of 2-AAF at a level almost comparable to that of the susceptible NIH-Swiss. Further studies on binding of the AAF to liver protein showed that the levels were also comparable in the two strains. Thus the marked difference in susceptibility of X/GF and Swiss mice to 2-AAF must rest on some other factor, perhaps the immunological status and not on the metabolic pattern.

3. Metabolism of 2-aminoanthraquinone in rats. In one of the bioassay contract studies (Mason) 2-aminoanthraquinone (2-AAQ) led to severe nephrotoxicity in female Fischer rats, due to deposition of 2-AAQ and a possible metabolite in the kidney tubules. Male animals were much less affected. Metabolism studies on 2-AAQ are now in progress, including studies of hormonal effects, to pinpoint the reasons for the difference.

4. High pressure liquid chromatography. Solvent programs for the high pressure liquid chromatograph are being developed to separate metabolites of various types of compound under study. Some, such as metabolites of 6-aminochrysene, are relatively insoluble, but can be separated well. The other extreme is 2,4-toluenediamine which is very soluble but has metabolites of widely varying polarity so that one program may not be sufficient to separate them.

5. *In vitro* study with liver cytosol from animals administered 2,4-toluenediamine. As part of our investigation of the metabolism of 2,4-toluenediamine (2,4-TDA), *in vitro* studies with 2,4-TDA, acetyl coenzyme A and liver cytosol from different species were undertaken. From all species and tissues examined hamster cytosol was most efficient in acetylating 2,4-TDA. Cytosols from guinea pig, rabbit, mouse and rat were effective in that order. Human liver had only a trace of activity while dog liver cytosol showed none. In all cases, the *p*-amino group of 2,4-TDA was selectively acetylated and only minor amounts of the *o*-acetyl derivative were formed.

6. Metabolism of 2,4-TDA in the rat. Following injection of ^{14}C-labeled 2,4-TDA in rats, the excretion of ^{14}C was fairly rapid and complete; 81% was recovered in 24 hours and almost 99% by 120 hours after injection. Urinary excretion was approximately 15%; fecal 22%. A relatively large fraction (22%) of urinary metabolites was unconjugated material; 7.5% glucuronic acid conjugates; 10% acid-hydrolyzable and 30% was water-soluble. The major unconjugated metabolites were 4-acetylamino-2-aminotoluene, 2,4-diacetyl-aminotoluene and 4-acetylamino-2-aminobenzoic acid. One of the glucuronide conjugated metabolites was 4-acetylamino-2-aminobenzoic acid. Radioactivity from the labeled 2,4-TDA was bound to nuclear DNA, to RNA, and to the microsomal and soluble proteins of liver.

Significance to Biomedical Research and the Program of the Institute:

By the utilization of certain chemical carcinogens, the molecular mechanism of steps leading to cancer is being explored. If the entire process is viewed as a series of steps from the primary interaction between an agent and a crucial cellular constituent or constituents, followed by multiplication of abnormal cells which in turn can undergo further transformations, there are a number of points where one could prevent or even reverse such interactions. Thus, the ultimate aim of these studies is to comprehend fully the sequence of complex reactions leading to cancer and eventually to be in a position to prevent them under realistic conditions in man.

Proposed Course of Project:

The program of elucidating the biochemistry of carcinogenesis, our eventual goal, is a continuing, long-term effort. Certain promising leads which have been discussed in this account will be followed up in further experiments.

Honors and Awards

Elizabeth K. Weisburger:

Member, Technical Committee, Society of Toxicology

Publications

Grantham, P. H., Mohan, L. C., Weisburger, E. K., Fales, H. M., Sokoloski, E. A. and Weisburger, J. H.: Identification of new water soluble metabolites of acetanilide. Xenobiotica 4: 69-76, 1974.

Poirier, L. A. and Weisburger, J. H.: Enzymic reduction of carcinogenic aromatic nitro compounds by rat and mouse liver fractions. Biochem. Pharmac. 23: 661-669, 1974.

Project No. Z01-CP-04620-10-CMT
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Metabolic Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mode of Action of Chemical Carcinogens -- Chemical Investigations

Previous Serial Number: Same

Principal Investigator: Elizabeth K. Weisburger, Ph.D.

Other Investigators: Timothy Benjamin, B.S., Nathan Lewis, B.S., and Thirayudh Glinsukon, Sc.D.

Cooperating Units: Analytical Chemistry Unit, CMT, NCI
Laboratory of Chemistry, NHLI
Viral Carcinogenesis Branch, NCI

Man Years:

Total	:	1.2
Professional	:	0.5
Other	:	0.7

Project Description

Objectives:

In order to gain an understanding of the etiology of cancer, chemical studies are performed on the properties of certain carcinogens. Model studies on the reactions of chemical carcinogens with potential targets are performed.

Methods Employed:

Properties of chemicals acquired or synthesized for the bioassay of chemical carcinogens are investigated to determine the purity of the materials. In model experiments the reactions of certain of these agents, particularly their primary and ultimate reactive forms, with select potential targets are studied. The materials produced are made available for related studies on the biochemistry and biology of the carcinogenic process. Methods for purification and analysis of various chemicals are applied.

Major Findings:

2,4-Toluenediamine metabolites. Various derivatives of 2,4-toluenediamine were prepared as reference materials for *in vivo* and *in vitro* metabolism studies of this industrial intermediate. Other carcinogens which are unavailable commercially are synthesized for other projects underway in the Branch.

Significance to Biomedical Research and the Program of the Institute:

The studies performed are a necessary adjunct to the broad programs on the mechanism of action of chemical carcinogens pursued in this Branch.

Proposed Course of Project:

This project will continue along the same lines as previously.

Publications

Glinsukon, T., Weisburger, E. K., Benjamin, T., and Roller, P. P.: Preparation and spectra of some acetyl derivatives of 2,4-toluenediamine. J. Chem. Eng. Data (in press).

Rhim, J. S., Park, D. K., Weisburger, E. K., and Weisburger, J. H.: Evaluation of an *in vitro* assay system for carcinogens based on prior infection of rodent cells with non-transforming RNA tumor virus. J. Nat. Cancer Inst. 52: 1167-1173, 1974.

Weisburger, E. K.: Industrial cancer risks. In Sax, N. Irving (Ed.): Dangerous Properties of Industrial Materials, 4th Edition, New York, Reinhold, 1975, pp. 274-288.

SUMMARY REPORT

CHEMISTRY BRANCH

July 1, 1974 through June 30, 1975

"Plans, develops and conducts a research program designed to (1) clarify the molecular biology of carcinogenesis; (2) elucidate the fundamental nature of the interactions of carcinogenic agents, especially chemical, with biological systems in the induction of cancer; (3) define those environmental and endogenous factors which relate to and modify the carcinogenic process; and (4) clarify the metabolic regulatory processes which are related to carcinogenesis."

The goal of the Chemistry Branch is to understand the molecular events of carcinogenesis with the view toward identifying susceptible populations and preventing human cancer. The research program is designed to understand the molecular basis by which carcinogenic agents cause malignant transformation and identify and characterize those exogenous and endogenous factors involved in carcinogenesis. The Branch seeks to clarify the metabolic interaction of exogenous agents with internal factors in the living organism at the molecular, cellular and host levels and seeks to understand the consequences of these interactions in terms of cell regulation and carcinogenesis. These processes are studied in cells from experimental animals and humans where possible.

Cancer is a disease in which the expression of genetic information is altered to give the tumor phenotype. This altered phenotypic expression may be due to genetic or epigenetic events induced by either xenobiotic or endogenous factors which result in altered patterns of gene control. The central aim of the Branch is to understand how exogenous carcinogens and endogenous factors are processed by enzymatic mechanisms, how the carcinogen is converted to active forms and the nature of the interaction between the latter and the gene action system. Understanding is sought in how this initial interaction results in the modifications which characterize malignant transformation. The aim of these studies is modification, elimination or prophylaxis of the carcinogenic response in the human population.

Senior Staff of this Branch is also responsible for the scientific direction of the Chemistry and Molecular Carcinogenesis Segment.

Cell Growth Regulation Section - *"Studies (1) the biochemical factors controlling cell growth and differentiation and the nature of the mechanisms governing the phenotypic expression of genetic information; (2) oncogenic and non-oncogenic viruses and other environmental agents capable of inducing alterations in growth behavior and differentiation."* The primary goal of this Section is to define biochemically the tumor cell and in particular the nature of the molecules responsible for converting a normal cell to its malignant form. One approach is to examine certain classes of molecules known to be involved in protein synthesis. Transfer-RNA (tRNA) functions in the assembly of the proper amino acids into the growing polypeptide chain.

Another role indicated for tRNA is that of regulation of protein synthesis in terms of the initiation, elongation and termination of the growing protein molecule. The majority of these studies in the past have been with biochemical systems derived from microorganisms. Analysis of mammalian tissues has revealed the regular occurrence of amino acyl-tRNAs which recognize termination (nonsense) codons. If these amino acyl-tRNAs suppress nonsense mutations, they may serve as a stabilizing protective mechanism against mutagens which induce such nonsense mutations. These mutagenic agents may be also carcinogenic. When amino acyl-tRNAs from different organs were compared, variations in virtually all 20 amino acyl-tRNAs were found.

Analysis of the codon responses of each of these fractionated aa-tRNAs demonstrated that the concentrations of specific isoacceptor aa-tRNAs for decoding genetic messages are different in different mammalian tissues, provides further evidence that the concentrations of isoacceptor aa-tRNAs may be related to the synthesis of specific proteins, and further substantiates the universality of the genetic code. Furthermore, an analysis of the transfer of amino acids from tRNAs which recognize specific codons into specific sites in protein provides a technique for identifying point mutations in proteins (e.g., in sickle cell anemia) for studying sequences of mRNA, as well as demonstrating which aa-tRNAs may be involved in modulating protein synthesis. These studies may provide a new understanding of the regulatory processes involved in carcinogenesis.

Studies to determine a physiologically active molecule primarily responsible for malignancy are underway. Certain virus (avian sarcoma viruses) produce a virus-gene product which induces malignant transformation in infected cells. Resolution of this virus-gene product and determination of its function would be important steps in elucidating the nature of malignant cells. Studies on the viral RNA of Rous sarcoma virus demonstrate that the size of this RNA is much less than formerly estimated, and that the gene products emanating from the viral RNA are limited enough for reasonable study. Analyses of virus mutants showed that none of the proteins involved in virus reproduction were responsible for malignant transformation. The elimination of these proteins from consideration allows a concentration of efforts to describe the "transformation protein" to a small number of residual virus-specified proteins. Mutants of Rous sarcoma virus have been isolated, and cells infected with a virus mutant act as tumor cells at one temperature and as normal cells at a higher temperature. The morphological changes characteristic of malignant transformation can be separated from several biochemical changes which occur as a result of transformation, and the primary physiological change can be studied in the absence of complicating secondary changes. Cells in the process of transformation increase in size, and their density, as measured in dextran density gradients, decreases. These changes can be observed in the absence of DNA, RNA, or protein synthesis, and can be explained by an observed increase in intracellular water. Coincident with an increase in cellular water is an increased accumulation of Na^+, which could in turn affect the regulation of cellular metabolism. In fact, manipulation of the ionic environment of non-transformed and transformed cells demonstrated that glucose metabolism critically depended upon the extracellular concentration of monovalent cations. Under investigation is the possibility that changes in intracellular water and ion concentrations are responsible directly for the induction and maintenance of malignancy after infection of cells by this virus.

Primary physiological changes in the malignant process must be related to other changes further along in the sequence of changes resulting in malignancy. Several inducible metabolic changes have been analyzed, including uptake of a variety of exogenous metabolites, synthesis of hyaluronic acid, and the synthesis of a large glycoprotein. These changes can be prevented under conditions where accumulation of water and Na^+ occur, and are thought to be secondary changes in this system. Such studies of cells transformed by Rous sarcoma virus offer the hope of a definition of the molecule primarily responsible for the physiological manifestation of malignancy, and the determination of metabolic changes necessary to the malignant process.

Molecular Carcinogenesis Section - *"Studies (1) the metabolic effects on tissues and cells resulting from their exposure to chemical carcinogens and the relationship between these effects and malignant transformation; (2) the metabolic activation and detoxification of the polycyclic hydrocarbons and the relationship of this metabolism to individual susceptibility."* This section studies the molecular events of malignant transformation induced by chemical carcinogens, in particular those of the polycyclic hydrocarbon class. The aim is to understand the enzymatic conversion of carcinogens to either detoxification forms or to the active carcinogenic metabolite. In parallel sutdies the interaction of the active metabolite with the relevant cellular receptor is being investigated. During evolution humans have been exposed to foreign chemical compounds (including carcinogens) and has developed metabolic systems for their detoxification and elimination. These systems primarily involve microsomal cytochrome P-450 mixed function oxygenases, but also include epoxide hydratases and conjugating enzymes. The vast majority of foreign compounds are processed by these enzyme systems. The mixed function oxygenases are influenced by a variety of environmental factors such as drugs, pesticides or carcinogens, and are influenced by the nutritional and hormonal state of the animal. The age, sex and genetic makeup also determine enzyme activity. Work in this laboratory provided the key studies which showed this enzyme system to be responsible for the activation of procarcinogens to their ultimate carcinogenic form. Thus it was shown that the mixed-function oxygenases were responsible for both the toxic and carcinogenic effects. This understanding of polycyclic hydrocarbon action was derived from five lines of evidence: (1) the toxicity of benzo[a]pyrene (BP) parallels the level of the enzyme in the cell; (2) the enzyme system catalyzes the formation of BP-DNA complexes; (3) the discovery of an inhibitor of the enzyme system (7,8-benzoflavone) which prevents BP cytotoxicity; (4) the inhibitor of the enzyme prevents the binding of BP to DNA, RNA and protein; and (5) the inhibition of the enzyme system reduces 7,12-dimthyl-benz[a]anthracene (DMBA) tumorigenicity by 90%.

A primary goal is to define the enzymatic mechanism by which polycyclic hydrocarbons are either activated to carcinogenic forms or to detoxified products. As these enzymes are characterized and sensitive methods are developed for their assay, it may be possible to characterize an individuals enzymatic makeup with respect to carcinogen metabolism and to understand the relationship between this metabolism and individual susceptibility to polycyclic hydrocarbon carcinogenesis. The known sequence of PCH metabolism is as follows: Oxygenation by the microsomal mixed function oxygenases one indicator of which is aryl hydrocarbon hydroxylase. Very sensitive methods

have been developed for the latter (see below) which are applicable to human tissues. Secondly epoxides are hydrated to dihydrodiols by oxide hydratase. The oxides are also metabolized by glutathione S-transferases. Sensitive assays (see below) for each of these systems as well as a method for total metabolite analyses by high pressure liquid chromatography (HPLC) have been developed.

Aryl hydrocarbon hydroxylase (AHH) has been analyzed in human monocytes. The conditions for optimum assay, reproducibility of the assay and variation in a small number of humans has been determined. Also the half-life of the AHH in humans was found to be about 4-5 hours. Various serums were found to give different maximum induction and inducibility in monocytes and lymphocytes has been compared. The monocytes have several advantages over the lymphocyte assay. These are that the fold induction is much greater (7-20 fold) compared to lymphocytes (2-4-fold) and the monocytes require no mitogen stimulation.

Further progress has been made in the development of high pressure liquid chromatography for the quantitative analyses of BP metabolites. The method has been applied to human liver and lymphocytes and at least 4-6 new BP metabolites have been detected. Further, a modification of the method has permitted the direct isolation and characterization of benzo[a]pyrene-4,5-oxide as a metabolite. In other studies the mechanism of action of two inhibitors of the enzyme complex 7,8-benzoflavone and trichloropropylene oxide have been elucidated. The former acts at the level of the mixed function oxidase and the latter primarily at the level of the arene oxide hydratase. This HPLC method in our laboratory is now being instituted in a large number of laboratories and is fast becoming the method of choice for studies of BP metabolism.

A method has been developed for the assay of the key enzyme benzo[a]pyrene 4,5-oxide hydratase, that converts the metabolite benzo[a]pyrene 4,5-oxide to the corresponding dihydrodiol. This enzyme has been characterized as to its properties, tissue distribution and variability in several mouse strains.

A method has been developed for the assay of glutathione-S-benzo[a]pyrene 4,5-oxide transferase. This is a key enzyme that conjugates the benzo[a]pyrene epoxide with glutathione. This may be a very important enzyme in the detoxification of the reactive intermediate. The properties of the enzyme have been defined with respect to kinetic behavior, pH optimum and sensitivity to various inhibitors.

In collaboration with the Department of Biochemistry, University of Michigan, several highly purified forms of the mixed function oxygenases, the cytochrome P-450 and P-448 have been analyzed for their activity toward BP with respect to product formation. Two preparations show no detectable diol formation which make them extermely useful for the study of BP metabolisms.

Studies in collaboration with the Cell Growth Regulation Section on the mechanism of induction have shown that the AHH induction is independent of ribosomal RNA synthesis since AHH induction occurs in temperature sensitive mutants with respect to ribosomal RNA synthesis.

Induction studies in near diploid rat liver epithelial cells have shown that AHH basal levels and inducibility are heterogenous within a cloned cell population. Thus, in cells derived from a common parent there exist heterogeneity. These findings have serious implications as to the uniformity of cells in vivo with respect to AHH and carcinogen activation as well as to transformation studies in vitro.

Further studies on the mechanism of butylated hydroxy toluene inhibition of carcinogenesis have indicated that this agent can modify the carcinogen metabolizing enzyme systems by both inducing the enzymes in liver and by inhibition in the target tissue.

Nucleic Acids Section - *"Studies (1) interaction of chemical carcinogens and their analogs with nucleic acids and with enzymes regulating nucleic acid metabolism; (2) the effect of these agents on the subcellular organization of nucleic acid macromolecules."* Three major areas of investigation are underway in the Nucleic Acids Section during the past year. One of these is the continued study of the subcellular and molecular events that follow the infection of susceptible cells, cultured in vitro, with RNA tumor viruses. Current work has indicated that the RNA-directed DNA copy of the viral genome is made in the cell cytoplasm in association with the plasma membrane and furthermore is a complete copy of the entire viral RNA genome. Concurrently two in vitro model systems (one employing mitochondria and another employing Colicin E1 DNA) for synthesizing DNA have been established in order to investigate the factors involved in the initiation of DNA replication.

In a second area of investigation, cellular and environmental factors involved in the malignant transformation of cells in vitro by chemicals are being elucidated. In a well-established mouse cell system it has been found that one cell generation is required to fix transformation while more than three cell generations are required for cells to express the transformed state. Caffeine, which inhibits post replication DNA repair, inhibits cell transformation by 4-nitroquinoline-1-oxide (4NQO), while acriflavin, which appears to inhibit excision repair, enhances cell transformation. Very encouraging have been some preliminary results which indicate that normal human cells, under strictly controlled conditions, can be transformed in vitro by both 4NQO and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

Finally, much effort is being directed to the study of DNA repair in human cells with special emphasis on the investigation of genotypes exhibiting defective DNA repair. Recent work has shown that normal human cells can repair DNA damage due to nitrous acid and mono-adducts of psoralen (induced by psoralen plus near-UV irradiation). Further, five different xeroderma pigmentosum variant cell strains, which by other techniques show normal repair of UV-induced DNA damage, have been found to have a DNA repair defect by means of a host-cell reactivation assay developed in this laboratory.

Protein Section - *"Studies (1) the relationship of proteins to the carcinogenic process; (2) interactions between carcinogenic agents and proteins; (3) changes in proteins occurring as a result of or concomitant with the*

administration of carcinogenic agents; relates the genetic control of such proteins to inheritance in laboratory animals and man." The Protein Section, as in the past, has been concerned with the development of better methods for the isolation and characterization of ribonucleic acids and proteins. In order to provide a sound understanding of these substances, which regulate growth and function in normal and malignant cells, they must be isolated, separated and chemically defined. The goal of this Section is to establish the means by which this can be accomplished, and to use this firm scientific background for studies in the nature of carcinogenesis.

(1) Conformational Studies of Nucleic Acids by Electrophoresis - Although the basic information used by the cell is contained in the linear array of bases in its DNA and subsequently in RNAs transcribed from it, the ability of nucleic acids to fold and assume various configurations is an important factor in the expression of this information. Numerous physical methods such as X-ray studies and thermal transitions have been used by workers in this field. Because many conformational states differ in their radius of gyration, it appeared possible that electrophoresis in polyacrylamide gels might serve to identify some of these conformational states and, in addition, to separate mixtures of such conformational isomers. The primary nucleic acids under study have been DNA, the ribosomal RNA species 18S and 28S, and transfer RNA. Systems were devised whereby these materials could be electrophoresed under a wide variety of salt conditions ranging from 1 mM to 1 M over a temperature range from 0 to 90°C. The most important finding was the tendency for all nucleic acids to adopt a rigid-rod conformation in reduced salt concentrations. The ability of the nucleic acids to pass through the sieving pores of the gel was strikingly reduced.

Of all transfer RNAs studied the initiator tRNA, f-met tRNA, was found to adopt a uniquely compact configuration at 50°. Whether this property is shared by initiator tRNAs from other species is under investigation.

On high resolution electrophoresis, ribosomal RNA species 28S and 18S from malignant cells and normal cells often are seen in multiple distinct bands. It has been found that by conversion of one band into another that these multiple bands within each species are conformational isomers. In addition to those isomers which are found by convertional extraction procedures, numerous other forms by laboratory manipulation involving divalent ions, heating, denaturants, etc. have been generated by this Section. The observed isomerism, therefore, argues against the previously stated hypothesis that the ribosomal RNA in tumors differed from that found in normal cells.

(2) Preparative Electrophoresis of Ribonucleic Acids - The high analytical resolution of gel electrophoresis has prompted many studies on adapting this method for preparative purposes. The ability to isolate a variety of messenger RNAs or nuclear RNAs possible with a control function would be of great significance. Although much effort has been devoted to this project in the past year, success to date is very limited; however, certain major problem areas have been defined. These include: (a) thermal gradients that develop as a result of the electric current, (b) the relatively small amount of nucleic acid that may be electrophoresed while preserving good separations,

(c) contamination of the product by components of the gels used, and (d) the handling of very dilute solutions of RNA and very tiny volumes of concentrated solutions of RNA.

(3) Characterization of Proteins and Other Molecules on Gels and in Histological Sections - Previous studies of the metachromatic reaction of the cationic carbocyanine dye, "Stains-all," with macromolecules have shown its usefulness for the demonstration of phosphoproteins, glycoproteins, and proteoglycans in deparaffinized tissue sections. Further studies with this dye using cryostat sections have demonstrated that the dye interacts with polar lipids as well. These were detected in mitochondria, the brain, the plexiform layers of the retina and on inner and outer surfaces of organs and tissues. Prior extraction of sections with lipid solvents prevented the staining due to lipid but preserved the staining of the remaining tissue components. An investigation of the staining of these substances on polyacrylamide gels has shown that sialic acid-rich glycoproteins stain differentially on gels. The specificity of the reaction for the acidic group was shown by the absence of the metachromatic reaction by prior digestion of the glycoprotein by neuraminidase. This method should be valuable in the identification of antigens involved in recognition processes where sialic acid-containing glycoproteins appear to play a major role. The methodology developed for the demonstration of phosphoproteins and sialic acid-rich glycoproteins in tissue sections and on acrylamide gels has been applied to normal and malignant mammary tissues of the mouse and has been used in the identification of the proteins from BALB/c and C3H mouse milk. Similar studies on human tissue are in progress.

Project No. Z01-CP-04520-04-CH
1. Chemistry Branch, Carcinogenesis, DCCP
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemistry of Transformation of Cells by Avian Sarcoma Viruses

Previous Serial Number: Same

Principal Investigators: John P. Bader, Ph.D. and Rudolf Harris, Ph.D.

Other Investigators: Artrice V. Bader*, Ph.D. and Nancy R. Brown

Cooperating Units: *Virus Studies Section, Viral Oncology, DCCP, NCI

Man Years:

Total	2.8
Professional	2.0
Other	0.8

Project Description

Objectives:

To delineate the basic physiological defect responsible for malignancy in Rous sarcoma virus-infected cells, and to determine the subsequent sequence of metabolic events resulting in the malignant state.

Methods Employed:

Cell culture, microscopic identification of cellular transformation, radioactive tracer techniques, polyacrylamide and agarose gel electrophoresis, electron microscopy, cell volume determinations, dextran density gradients.

Major Findings:

Morphological transformation induced in cells by Rous sarcoma virus (RSV-BH) was found to be accompanied by an increase in size, due in part to an increased accumulation of water. An increase in uptake of Na^+ also was found in transformed cells. In this lab, mutants of RSV-BH have been isolated which induce temperature-dependent transformation in infected cells; infected cells behave as transformed cells at 37°, but have a nontransformed phenotype at 41°. When

such cells are grown at 41° then shifted to 37°, an increase in size and intracellular water occurs, and these changes can occur without new DNA, RNA, or protein synthesis. Likewise, Na^+ uptake increased after the temperature shift.

The possibility that alterations in intracellular water and Na^+ could affect inducible enzymes by altering RNA synthesis is under investigation. In order to do this, one inducible system, an increased capacity for glucose uptake, was characterized in detail. The increased capacity for glucose uptake is prevented by Actinomycin D, high adenosine concentrations, cordycepin, cycloheximide, or puromycin, i.e., substances which inhibit RNA or protein synthesis. Kinetic analyses showed that both V_{max} and K_m changed during induction, and inhibition of RNA or protein synthesis prevented changes in both V_{max} and K_m. These results suggested that new uptake sites were synthesized in transformed cells.

Nontransformed cells also attain an increased capacity for glucose uptake if deprived of exogenous glucose for several hours. The V_{max} and K_m for glucose uptake change in these cells, and such changes are prevented by inhibitors of RNA or protein synthesis. The possibility that transformed cells increase the capacity for glucose uptake by failing to repress the inducible sites was examined by incubating transformed and nontransformed cells in varying concentrations of glucose. Large differences in uptake capacity were found only in nontransformed cells, i.e., the transformed cells were relatively unresponsive to repression by glucose.

Changing the pH of the growth medium was found to induce substantial changes in glucose uptake capacity. In these experiments, the V_{max} changed without alteration in K_m, and neither Actinomycin D nor cycloheximide prevented the changes. These pH-induced changes occurred in both nontransformed and transformed cells, and were concluded to be irrelevant to the transformation process.

Cytoplasmic vacuolization is another characteristic of cells transformed by RSV-BH, and this vacuolization may be a cellular response to increased water and Na^+ uptake. A knowledge of the vacuolization process could reveal important features of transformation. Histological staining showed the vacuoles contained little or no macromolecules, although the uptake of neutral red into the vacuoles of viable cells showed that water was a major constituent. The vacuoles are bound by membrane and appear to arise from within the cytoplasm. Vacuolization requires only Na^+ as cation in the solution and Na^+ cannot be replaced by Li^+. Clearly vacuolization is related to other observed changes in transformed cells suggesting a role for water and Na^+ in transformation.

Significance to Biomedical Research and the Program of the Institute:

These are the first recorded attempts to define a physiological basis for malignant cells: to determine the primary physiologically active molecule which is altered, and to establish a sequence of metabolic changes occurring in the malignant process. Such studies may lead to a determination of metabolic events necessary to the maintenance of the malignant state, but not to

normal metabolism, and the possibility of therapeutic intervention in such metabolism.

Proposed Course of Project:

Resolution of primary and secondary changes in the metabolism of transformed cells. To examine the role of monovalent cations in the regulation of RNA synthesis. To further characterize cytoplasmic vacuoles in the possibility that these represent specialized organelles functioning in maintaining water and ion balances. To attempt to identify virus-specified proteins responsible for transformation.

Honors and Awards

Invited speaker, Symposium on Mechanisms of Virus Disease, Squaw Valley, California, March, 1974.

Invited speaker, Symposium on Fundamental Aspects of Neoplasia, Rutgers.the State University, New Brunswick, New Jersey, May, 1974.

Invited speaker, Symposium on Carcinogenesis In Vitro, Annual Meeting of the Tissue Culture Association, Miami, Florida, June, 1974.

Guest lecturer, The Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina, September, 1974.

Guest lecturer, Harvard Medical School, Boston, Massachusetts, December, 1974.

Publications

Bader, J. P.: Conditional transformation of cells infected with a mutant of Rous sarcoma virus. In Ts'o, P. O. P. and DiPaolo, J. A. (Eds.): Chemical Carcinogenesis. New York, Marcel Dekker, Inc., 1974, pp. 661-668.

Bader, J. P.: Reproduction of RNA tumor viruses. In Fraenkel-Conrat, H. and Wagner, R. R. (Eds.): Comprehensive Virology. New York, Plenum Press, 1974, pp. 253-332.

Clark, J. M. and Bader, J. P.: Inhibition of development of transformation by Rous sarcoma virus in cultures of high cell density. J. Cell. Physiol. 83: 203-210, 1974.

Bader, J. P. and Bader, A. V.: Characteristics of cells transformed by Bryan Rous sarcoma virus. In Robinson, W. S. and Fox, C. F. (Eds.): Mechanisms of Virus Disease. Menlo Park, California, W. A. Benjamin, Inc., 1974, pp. 303-313.

Bader, J. P., Ray, D. A. and Brown, N.R.: Accumulation of water during transformation of cells by an avian sarcoma virus. Cell 3: 307-313, 1974.

Project No. Z01-CP-04557-02-CH
1. Chemistry Branch, Carcinogenesis, DCCP
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Chromatographic and Codon Response Differences in the Amino Acyl-tRNAs of Mammalian Tissues (An Analysis of the Genetic Code in Mammalian Cells)

Previous Serial Number: None

Principal Investigators: Dolph Hatfield, Ph.D., M. Caicuts and C. Robert Matthews*

Other Investigators: None

Cooperating Units: None

Man Years:

Total	2.2
Professional	1.2
Other	1.0

Project Description

Objectives:

To detect chromatographic differences in the AA-tRNAs of different mammalian tissues and to determine codon responses of isoaccepting species of AA-tRNAs from different mammalian tissues.

Methods Employed:

Transfer RNA and AA-tRNA synthetases are isolated from rabbit liver and rabbit reticulocyte-rich blood and from bovine liver and brain. Chromatographic differences are detected by comparing AA-tRNAs from one tissue to those of a second tissue by reverse phase chromatography. Large scale preparation of AA-tRNAs from each tissue are then resolved by reverse phase chromatography and individual isoaccepting species of AA-tRNAs are pooled and assayed for codon recognition by the binding assay of Nirenberg and Leder (Science 145: 1399, 1964).

* A portion of this work was carried out when C. Robert Matthews (California Institute of Technology) was visiting the National Cancer Institute.

Major Findings:

Chromatographic differences were observed in the elution profiles of virtually all 20 AA-tRNAs of rabbit liver and rabbit reticulocytes (see also Smith and McNamara, Biochem. Biophys. Acta 349: 366, 1974). Chromatographic differences were observed in the elution profiles of 6 of 20 AA-tRNAs of bovine liver and brain. Codon responses of isoaccepting species of AA-tRNAs from rabbit liver and reticulocytes and of several AA-tRNAs of bovine liver and brain were determined. These studies have: 1) demonstrated that the levels of some isoaccepting species of AA-tRNA which recognize a specific codon are present in greater quantities in some tissues than in others (e.g., the seryl-tRNA which recognizes the codon UCG is more pronounced in rabbit liver than in reticulocytes and the valyl-tRNA which recognizes GUG is more pronounced in bovine liver than brain); 2) provide further evidence that the concentrations of isoacceptor AA-tRNAs may be specialized, at least in reticulocytes, for protein synthesis, and 3) further substantiate the universality of the genetic code.

Significance to Biomedical Research and the Program of the Institute:

A major unresolved question in biology is whether tRNA may play a role in cellular regulation and carcinogenesis. One approach to elucidating these problems is a study of the tissue specificity of AA-tRNAs.

Proposed Course of Project:

The project is completed.

Project No. 701-CP-04587-01-CH
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Cell Growth Regulation
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Incorporation of Amino Acyl-tRNAs into Specific Sites of Rabbit Globin

Previous Serial Number: None

Principal Investigators: Dolph Hatfield, Ph.D., LeRoy Richer*, Ph.D., D. W. E. Smith+, M.D. and M. Caicuts

Other Investigators: None

Cooperating Units: *Department of Chemistry, California Institute of Technology and +Department of Pathology, Northwestern University Medical School

Man Years:

Total	3.2
Professional	2.1
Other	1.1

Project Description

Objectives:

To provide a simple assay for methionyl-tRNA$_f$ and methionyl-tRNA$_m$ and to determine the levels and sites of incorporation of these isoacceptors into rabbit globin. Additionally, to determine which isoacceptors of arginyl-tRNA are used to code for arginine in rabbit globin and to determine if any of these amino acid sites are modulators of globin synthesis.

Methods Employed:

Methionyl-tRNA$_f$ and methionyl-tRNA$_m$ from rabbit reticulocytes and from E. coli were resolved by reverse phase chromatography. Purified methionyl-tRNA$_f$ from E. coli is a commercial product. These isoacceptors were added to a rabbit reticulocyte lysate and their rates of incorporation into globin were determined. Large scale preparations of labeled globin were made from each isoacceptor methionyl-tRNA and the resulting α and β chains of globin separated by CM-cellulose. Following trypsin digestion, the peptides were resolved on a Dowex 50 Column. Each isoacceptor of arginyl-tRNA is presently being resolved

by reverse phase chromatography. The purified isoacceptor will be incorporated into rabbit globin. The α and β chains of rabbit globin will then be separated by CM-cellulose, digested with trypsin and the resulting peptide identified.

Major Findings:

Greater than 90% of the methionine was removed from rabbit reticulocyte methionyl-tRNA$_f$ during incubation with the reticulocyte lysate and the methionine was not incorporated into globin. However, greater than 90% of methionyl-tRNA$_m$ from reticulocytes and from E. coli and approximately 30% of the methionine from E. coli methionyl-tRNA$_f$ was incorporated into globin. Each methionyl-tRNA was incorporated into αT5 and βT5 (internal positions) and not into αT1 or βT1 (terminal positions). The studies with arginyl-tRNA are in the preliminary stages.

Significance to Biomedical Research and the Program of the Institute:

A major unresolved question in biology is whether tRNA may play a role in cellular regulation and carcinogenesis. One approach to elucidating these problems is to study the incorporation of specific isoacceptor AA-tRNAs into globin and to identify modulating AA-tRNAs.

Proposed Course of Project:

To identify which isoacceptors of arginyl-tRNA code for arginine in rabbit globin. The studies with methionyl-tRNA are completed.

Project No. ZO1-CP-04593-01-CH
1. Chemistry Branch, Carcinogenesis, DCCP
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cell-Surface Membranes of Virus-Transformed and Nontransformed Cells

Previous Serial Number: None

Principal Investigators: John P. Bader, Ph.D., Takumi Okazaki, M.D., and Dante Marciani, Ph.D.*

Other Investigators: Colin Chignell, M.D.+

Cooperating Units: *Laboratory of Biochemistry, DCBD, NCI, and +Laboratory of Chemical Pharmacology, NHI

Man Years:

Total	2.4
Professional	2.2
Other	0.2

Project Description

Objectives:

To characterize cell-surface membranes of virus-transformed and nontransformed cells, in order to determine if specific differences in cell surfaces can be related to altered growth properties of transformed cells.

Methods Employed:

Cell culture, transformation of cells by avian sarcoma viruses, radioactive labelling, isolation of cell-surface membranes by density gradient techniques, polyacrylamide gel electrophoresis, electron spin resonance techniques.

Major Findings:

Cell-surface membranes prepared by density banding of membrane vesicles presented characteristic patterns when proteins were separated by electrophoresis in polyacrylamide gels. No differences were found between virus-transformed and nontransformed cells, suggesting that no major alterations in cell-surface membranes *per se* occurred as a result of transformation.

When membranes were fixed with Zn^{++} before cellular disruption to retain loosely attached molecules, decreases in large glycoproteins were commonly observed in preparations from transformed cells. A mutant virus, RSV-BH-Ta, induces temperature-dependent transformation in infected cells, and such cells were used to analyze the glycoprotein differences. Upon shifting cells from the nontransforming temperature (41°) to the transforming temperature (37°) obvious morphological changes, as well as changes in glucose and hyaluronic acid metabolism, occurred prior to any obvious decrease in large glycoprotein. When cells were shifted from 37° to 41° and transformation was reversed, accumulation of large glycoprotein occurred later. Also, reversal of morphological transformation could occur without RNA or protein synthesis, processes required for the accumulation of large glycoprotein. It appears that the detachment of this glycoprotein(s) from the cell-surface is a late event in transformation, and cannot be responsible for several biophysical or metabolic changes observed in transformed cells.

The surface membranes of transformed and nontransformed cells were examined for differences in stability by electron spin resonance. Using lipophilic probes, no differences in membranes of nontransformed and cells transformed by several RNA or DNA viruses could be detected.

Significance to Biomedical Research and the Program of the Institute:

Increased fluidity of cell-surface membranes, and loss of cell-surface glycoprotein, are two popular hypotheses explaining malignancy of transformed cells. Neither of these hypotheses is supported by our experiments, and if changes in the cell-surface membrane are determining features of malignant cells, other changes, and other methods for detecting such changes, must be employed.

Proposed Course of Project:

To examine other features of cell-surface membranes (e.g. phosphorylated proteins, Na^+ K^+ ATPase), and to analyze their relevance in the induction and maintenance of the malignant state.

Publications

Marciani, D. J. and Bader, J. P.: Polypeptide composition of cell membranes from chick embryo fibroblasts transformed by Rous sarcoma virus. Biochim. Biophys. Acta (in press).

Bader, J. P. and Marciani, D. J.: Characteristics of cells transformed by Rous sarcoma virus. In Gottlieb, A. A. (Ed.): Fundamental Aspects of Neoplasia. New York, Springer-Verlag (in press).

Project No. Z01-CP-04748-07-CH

1. Chemistry Branch, Carcinogenesis, DCCP
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Synthesis and Characterization of the RNA of RNA-containing Tumor Viruses

Previous Serial Number: Same

Principal Investigator: John P. Bader, Ph.D.

Other Investigators: David A. Ray

Cooperating Units: None

Man Years:

Total	1.3
Professional	0.3
Other	1.0

Project Description

Objectives:

To determine the molecular structure of the RNA of RNA-containing tumor viruses and to elucidate the intracellular site and mechanism of synthesis of viral RNA.

Methods Employed:

Cell culture, radioactive tracer techniques, density-equilibrium and sedimentation rate centrifugations, isolation and purification of nucleic acids, polyacrylamide gel electrophoresis, spectroscopy, enzymatic degradations.

Major Findings:

The RNA of RNA-containing tumor viruses has been examined by biophysical and biochemical techniques in attempts to characterize the RNA, to relate the structure of the RNA to its unique capacity to initiate and specify complementary DNA, and to determine the informational content of the viral genome. The apparent high molecular weight native RNA, as extracted from virions, can be denatured by agents which dissociate hydrogen bonds into molecules of molecular weight 2.5 - 3.0 x 10^6 daltons. The usual interpretation of these observations is that the native viral RNA has a molecular weight of 9 x 10^6 daltons and is composed of 3 or 4 subunits of lower molecular weight.

Possible aggregation of low molecular weight molecules during the extraction procedure was eliminated as a possibility for the formation of high molecular weight RNA. Attempts to renature partially denatured molecules were uniformly negative, and were consistent with earlier observations on the interspersion of intramolecular double-stranded regions. Incomplete denaturation of the extracted native RNA produced transitional forms; no discrete species indicative of subunit aggregates which had lost one or more subunits were found. The most reasonable explanation for the data is that native viral RNA has a configuration which affects its hydrodynamic properties. Since the methods used to estimate molecular weight depend upon hydrodynamic properties, such estimates are probably erroneous. Native viral RNA probably exists as a monomer of molecular weight 2.5 - 3.0 x 10^6 daltons which changes its configuration upon denaturation.

Significance to Biomedical Research and the Program of the Institute:

Estimates of the number and size of virus-specified proteins can be related to the informational content (size) of the viral RNA. Knowledge of the viral proteins required for virus production allows one to estimate the amount of residual genetic information available to make polypeptide responsible for malignant transformation. If the above interpretation is correct, and viral RNA is 2.5 - 3.0 x 10^6 million daltons, residual RNA can code for polypeptide of less than 40,000 daltons. The possibility that a virus-specific physiological molecule responsible for malignant transformation can be resolved then becomes feasible.

Proposed Course of Project:

To determine unequivocally the molecular weight and genetic content of intravirion RNA. To initiate experiments to detect virus-specified protein related to transformation, but different from virus structural proteins, in an attempt to define the physiologically active molecule primarily responsible for transformation by an avian sarcoma virus.

Project No. Z01-CP-04522-04-CH
1. Chemistry Branch, Carcinogenesis, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Molecular Mechanisms of Aryl Hydrocarbon Hydroxylase Induction in Mammalian Cell Culture

Previous Serial Number: Same

Principal Investigator: Harry V. Gelboin, Ph.D.

Other Investigators: James P. Whitlock, Jr., M.D. Hayden G. Coon, Ph.D. Dr. H. Aviv and Dr. C. Prives

Cooperating Units: Chemistry Department, Weisman Institute

Man Years:

Total	:	0.8
Professional	:	0.4
Other	:	0.4

Project Description

Objectives:

To understand the biochemical mechanisms of regulation of the AHH system in cells cloned from rat liver.

Methods Employed:

Cell culture; spectrophotofluorometry; recording spectrophotometry; agarose-polyacrylamide gel electrophoresis; radioactivity measurements by scintillation counting.

Major Findings:

There is substantial variation in basal and induced hydroxylase activity in clones propagated from a parental strain in which the expression of hydroxylast activity has remained stable.

Attempts are being made to induce hydroxylase systems in a cell free preparation using messenger enriched RNA as a primer.

Significance to Biomedical Research and the Program of the Institute:

AHH, a microsomal mixed-function oxygenase, is found in most tissues of many species, including humans, and plays an important role in both the detoxification and activation of carcinogenic polycyclic hydrocarbons. An understanding of the control of this enzyme complex is relevant both to chemical carcinogenesis and drug metabolism and to the problem of biochemical events in the regulation of enzyme induction.

Proposed Course of Project:

This system is useful for the study of microsomal hydroxylase, for the study of the effect of polycyclic hydrocarbons on the cell, and for the study of the molecular biology of hydroxylase induction.

Project No. Z01-CP-04533-03-CH
1. Chemistry Branch
Carcinogenesis, DCCP
2. Molecular Carcinogenesis
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Kinetics of Product Formation During Polycyclic Hydrocarbon Metabolism

Previous Serial Number: Same

Principal Investigator: James K. Selkirk, Ph.D. and Shen K. Yang, Ph.D.

Other Investigators: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total	:	0.5
Professional	:	0.2
Other	:	0.3

Project Description

Objectives:

Determination of rate of formation of products. Determination of precursor-product relationship as well as the relationship between phenol and dihydrodiol, i.e. hydration vs. dehydration of the various metabolites.

Methods Employed:

High-pressure liquid chromatography; fluorescence assay.

Major Findings:

MCA-induced liver microsomes metabolize BP to 3-hydroxy-BP at zero order kinetics for about 10 minutes as measured by fluorescence assay. At this time the metabolite profile is constant up to 30 minutes and it has been possible to selectively inhibit diol formation using enzyme inhibitors.

Significance to Biomedical Research and the Program of the Institute:

There are a number of similar products formed during metabolism of benzo(a)pyrene. It is not yet known which of these derive from the activated carcinogenic state and which ones are from other intermediates.

Proposed Course of Project:

Incubations at shorter times will indicate the chronology of metabolite appearance and the preferential metabolic pathway will be determined.

Publications

Selkirk, J.K., Croy, R.G. and Gelboin, H.V.: High-pressure liquid chromatographic analysis of benzo(a)pyrene metabolism and covalent binding and the mechanism of action of 7,8-benzoflavone and 1,2-epoxy-3,3,3-trichloropropane. Cancer Res. 34: 3474-3480, 1974.

Project No. Z01-CP-04534-01-CH
1. Chemistry Branch
Carcinogenesis, DCCP
2. Molecular Carcinogenesis
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Separation of Polycyclic Hydrocarbon Metabolites by High-Pressure Liquid Chromatography

Previous Serial Number: Same

Principal Investigator: James K. Selkirk, Ph.D.

Other Investigators: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total	:	0.8
Professional	:	0.4
Other	:	0.4

Project Description

Objectives:

To rapidly and quantitatively separate the known derivatives of carcinogenic polycyclic hydrocarbons and to attempt to maximize the efficiency of separation by variance of column packing, solvent and temperature while maintaining molecular structure.

Methods Employed:

Dupont 830, high-pressure liquid chromatograph, chromatronix 3500, liquid chromatograph.

Major Findings:

All labile functional groups (e.g. epoxy, hydroxyl) must be protected and must be able to be volatilized in order to utilize gas chromatography. In the case of polycyclic hydrocarbons, volatilizing temperatures are usually too close to the decomposition point to adequately separate polycyclic hydrocarbon derivatives. These compounds when injected can pass through this liquid chromatography system without decomposition. Eight metabolites of BP have been isolated and characterized by this system. Recycling

techniques have been utilized to separate compounds with structural characteristics too closely related for adequate separation under standard LC conditions. The system has been modified to isolate and identify an epoxide and a metabolic intermediate.

Significance to Biomedical Research and the Program of the Institute:

The product profile of polycyclic hydrocarbon metabolism indicates some relationship between the various products formed and activity as a carcinogen. A rapid analytical technique will allow us to determine which metabolic route is the most related to carcinogenicity and which to detoxification. Rapidity of analysis will lessen the chance of oxidation or other non-metabolic breakdown product formation and give a more accurate picture of the system in its in vivo state.

Publications

Selkirk, J.K., Croy, R.G., and Gelboin, H.V.: Benzo(a)pyrene metabolites: Efficient and rapid separation by high-pressure liquid chromatography. Science 184: 169-171, 1974.

Selkirk, J.K., Croy, R.G., and Gelboin, H.V.: Isolation by high-pressure liquid chromatography and characterization of benzo(a)pyrene-4,5-epoxide as a metabolite of benzo(a)pyrene. Arch. Biochem. Biophys. (in press).

Project No. Z01-CP-04556-03-CH
1. Chemistry Branch
Carcinogenesis, DCCP
2. Molecular Carcinogenesis
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Role of Sulfhydryl Groups in Polycyclic Hydrocarbon Metabolism and Carcinogenesis

Previous Serial Number: Same

Principal Investigator: James K. Selkirk, Ph.D.

Other Investigators: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total	0.8
Professional	0.4
Other	0.4

Project Description

Objectives:

To determine the relationship of sulfhydryl binding of carcinogens to the metabolism and carcinogenesis of polycyclic hydrocarbons. The binding of sulfhydryl compounds such as glutathione renders the conjugate water soluble. It is planned to investigate the kinetics of binding of polycyclic hydrocarbons to glutathione in mouse skin.

Methods Employed:

Enzymology, spectrofluorometry, mouse skin tumorigenesis, and metabolite analysis.

Major Findings:

A series of thiophosphoric esters and disulfides used as radiation agents reduce skin tumorigenesis in mice.

Significance to Biomedical Research and the Program of the Institute:

Conjugation of toxic substances to metabolites such as glutathione or glucuronic acid are common pathways for drugs and other foreign organic chemicals taken into the living organism. If the lifetime of the active form of the carcinogen is determined by the rate of its conjugation to a nontoxic product, then acceleration of the reaction should reduce the half-life of the proximate carcinogen and consequently the formation of tumors.

Proposed Course of Project:

To determine the effect of these reagents upon the drug metabolizing enzymes and the metabolite pattern of benzo(a)pyrene.

Project No. Z01-CP-04559-02-CH
1. Chemistry Branch
Carcinogenesis, DCCP
2. Molecular Carcinogenesis
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Benzo(a)pyrene-4,5-oxide Hydratase

Previous Serial Number: Same

Principal Investigators: Janet C. Leutz and Harry V. Gelboin, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	1.0
Professional	:	0.1
Other	:	0.9

Project Description

Objectives:

To increase the sensitivity of the assay for benzo(a)pyrene-4,5-oxide (BPO) hydratase. To study the properties of BPO hydratase in liver and extrahepatic tissues of rodents. To measure BPO hydratase activity in target tissues for carcinogenic polycyclic aromatic hydrocarbons such as skin and lung and in readily available tissues of humans such as white blood cells.

Methods Employed:

Thin layer chromatography, high pressure liquid chromatography, U.V. spectral analysis, spectrofluorometry.

Major Findings:

A radiometric assay utilizing thin layer chromatography has been developed to measure enzymatic hydration of BPO. The amount of benzo(a)pyrene-4,5-dihydro-4,5-diol product formed is linear with incubation time and protein concentration. The enzyme has no requirement for oxygen, NADPH, or magnesium, and it is inhibited by 1.1.1-trichloropropylene oxide. It is highly resistent to protease digestion unless it is preincubated first with mild detergent. The

hepatic enzyme is inducible by in vivo pretreatment with phenobarbital but not with 3-methylcholanthrene. BPO hydratase is present in all hepatic and extrahepatic tissues tested including mouse skin, rat mammary gland, human monocytes and cells in culture.

Significance to Biomedical Research and the Program of the Institute:

Following metabolic activation, BP binds to DNA, RNA, and protein. It is currently thought that oxide intermediates may be the activated form of BP which binds to macromolecules. The BPO hydratase reaction is one of removal of BPO from an animal system.

Proposed Course of Project:

To investigate the relationship of the enzyme level to tumor susceptibility in various strains of mice. To determine the enzyme level in readily obtainable human tissues.

Publications

Leutz, J.C. and Gelboin, H.V.: Benzo(a)pyrene-4,5-oxide hydratase: Assay, properties and induction. Arch. Biochem. Biophys. (in press).

Project No. Z01-CP-04565-02-CH
1. Chemistry Branch
   Carcinogenesis, DCCP
2. Molecular Carcinogenesis
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Comparative Studies of Rodent Liver Metabolism of Benzo(a)pyrene

Previous Serial Number: Same

Principal Investigator: James K. Selkirk, Ph.D.

Other Investigators: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total	:	0.5
Professional	:	0.2
Other	:	0.3

Project Description

Objectives:

To measure the metabolite profile of benzo(a)pyrene in mouse, rat and hamster liver. Also, to compare control liver to methylcholanthrene-treated liver.

Methods Employed:

High-pressure liquid chromotagraphy.

Major Findings:

While rat and mouse show relatively the same pattern, hamster liver shows more selective dihydrodihydroxy production. Induced liver shows marked increase in dihydrodihydroxy production.

Significance to Biomedical Research and the Program of the Institute:

Since there is a species susceptibility to tumor formation by polycyclic hydrocarbons, it is important to determine which are the preferential metabolic pathways in susceptible and refractory species.

Proposed Course of Project:

To determine if there are differences in rate of formation using new high-pressure liquid chromatography recycling methods and inhibition of oxidase and hydrase enzymes.

Project No. 701-CP-04588-01-CH
1. Chemistry Branch
Carcinogenesis, DCCP
2. Molecular Carcinogenesis
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Development of an Assay for Glutathione (GSH)-S-Transferase - Polycyclic Hydrocarbon Oxides

Previous Serial Number: None

Principal Investigator: Nobuo Nemoto, Ph.D.

Other Investigator: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total	1.2
Professional	1.2
Other	

Project Description

Objectives:

Examination of GSH-S-transferase using chemical carcinogens as substrates.

Methods Employed:

Fractionation of various organs, thin layer chromatography.

Major Findings:

Sensitive assay for GSH-S-Benzo(a)pyrene-4,5-epoxide transferase is established. The properties of this enzyme have been characterized.

Significance to Biomedical Research and the Program of the Institute:

The enzyme has an implication in the detoxification of chemical carcinogens. To compare the activity in various tissues would contribute to understanding the mechanism of their sensitivity to chemical carcinogens.

Proposed Course of Project:

Examination of GSH-S-BP-4,5-epoxide transferase activity in organs of several kinds of mouse strains and in human cells in culture. To make clear the metabolism of polycyclic hydrocarbon in activation and detoxification pathways.

Project No. Z01-CP-04589-01-CH
1. Chemistry Branch
Carcinogenesis, DCCP
2. Molecular Carcinogenesis
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Metabolism of Monohydroxy-benzo(a)pyrenes

Previous Serial Number: None

Principal Investigators: Friedrich J. Wiebel, M.D. and Haywood L. Waters

Other Investigators: James K. Selkirk, Ph.D. and Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total	0.6
Professional	0.3
Other	0.3

Project Description

Objectives:

To study the metabolism of monohydroxylated benzo(a)pyrenes, major products of microsomal metabolism of benzo(a)pyrene (BP). To study the interaction of BP-metabolism with that of its phenolic products.

Methods Employed:

Cell culture techniques, fluorospectrophotometry, recording spectrophotometry, high-pressure liquid chromatography.

Major Findings:

The 3-, 6- and 9-monohydroxy-benzo(a)pyrenes (HO-BP) are metabolized by microsomes from rat liver in vitro. The following findings indicate that the HO-BP's are metabolized by mixed-function oxygenases which are similar if not identical to the "benzo(a)pyrene hydroxylases": 1) the requirement for NADPH and the inhibition by carbon monoxide, 2) the microsomal localization, 3) the rates of reaction, 4) the inducibility of activity by 3-methylcholanthrene, and 5) the in vitro inhibition or stimulation by benzoflavones in microsomes from 3-methylcholanthrene treated or untreated animals. In mammalian cell cultures 3-HO-BP is converted by a mechanism different from that in hepatic microsomes.

Significance to Biomedical Research and the Program of the Institute:

The measurement of the aryl hydrocarbon (BP) hydroxylases is influenced by the further microsomal metabolism of the phenolic products and by their possible interference with the primary hydroxylation reaction. Since HO-BP's are highly cytotoxic and may be carcinogenic, it is important to understand their metabolic conversion.

Proposed Course of Project:

To identify the multiple products of HO-BP metabolism by microsomes and mammalian cells in culture and to elucidate their role in cytotoxicity and tumorigenicity.

Publications

Wiebel, F.J.: Metabolism of Monohydroxy-benzo(a)pyrenes by Rat Liver microsomes and Mammalian Cells in Culture. Arch. Biochem. Biophys. (in press).

Project No. Z01-CP-04590-01-CH
1. Chemistry Branch
Carcinogenesis, DCCP
2. Molecular Carcinogenesis
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Polycyclic Hydrocarbon Metabolism by Multiple, Purified Forms of Cytochrome P-450

Previous Serial Number: None

Principal Investigators: Friedrich J. Wiebel, M.D., Harry V. Gelboin, Ph.D., James K. Selkirk, Ph.D. and Janet C. Leutz

Other Investigators: David A. Haugen, Theodore A. van der Hoeven and Minor J. Coon

Cooperating Units: Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan

Man Years:

Total	1.0
Professional	0.6
Other	0.4

Project Description

Objectives:

To determine the metabolism of carcinogenic polycyclic hydrocarbons by purified forms of cytochrome P-450, the principal enzymes involved in carcinogen activation and detoxification. To study their role in polycyclic hydrocarbon toxicity, mutagenicity and tumorigenicity.

Methods Employed:

Fluorospectrophotometry, recording spectrophotometry, high pressure liquid chromatography, column chromatography.

Major Findings

The metabolism of benzo(a)pyrene by four highly purified forms of cytochrome P-450 from rabbit liver has been studied. The multiple forms of the cytochrome differed strongly in their total catalytic activity and in the profiles of oxygenated products from BP. Incubation of BP with 3 of the 4 preparations

led to the formation of BP-phenols, and -quinones, but not of dihydrodiols. Direct measurement of BP-4,5-oxide hydration confirmed the absence of hydratase activity in these purified preparations.

Significance to Biomedical Research and the Program of the Institute:

The microsomal mixed function oxygenases, which contain as their terminal electron acceptor the cytochromes P-450, are key factors in the response of organism to potentially toxic and carcinogenic foreign compounds. The analysis of the specificity of multiple P-450 forms is an essential step toward the understanding of chemical carcinogen action, and may ultimately help in the modification of the body's defense against chemical carcinogens.

Proposed Course of Project:

To identify polycyclic hydrocarbon metabolites formed by the multiple forms of cytochrome P-450 from various species. To determine the role of the enzyme forms in the biological effects of polycyclic hydrocarbons in cytotoxicity, mutagenicity and carcinogenicity.

Project No. Z01-CP-04591-01-CH
1. Chemistry Branch
Carcinogenesis, DCCP
2. Molecular Carcinogenesis
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Analysis of Cutaneous Enzymes Involved in Polycyclic Hydrocarbon Metabolism

Previous Serial Number: None

Principal Investigators: Friedrich J. Wiebel, M.D., Harry V. Gelboin, Ph.D. and Janet C. Leutz

Other Investigators: None

Cooperating Units: None

Man Years:

Total	0.6
Professional	0.3
Other	0.3

Project Description

Objectives:

To study the enzymes from mouse skin involved in polycyclic hydrocarbon metabolism. To elucidate their role in cutaneous polycyclic hydrocarbon tumorigenesis.

Methods Employed:

Fluorospectrophotometry, recording spectrophotometry, histological techniques, mouse skin tumorigenesis.

Major Findings:

Mouse skin contains aryl hydrocarbon (benzo(a)pyrene) hydroxylase activity which is inducible by aromatic polycyclic hydrocarbons and benzoflavones. The cutaneous hydroxylase activity is inhibited by carbon monoxide and requires the presence of NADPH indicating that the enzyme is one of the mixed-function oxygenases. The highest enzyme activity was found in the superficial layer of skin which contains the sebaceous glands and the

upper pilary canals. Enzyme activities were intermediate in the epidermis and lowest in the deeper dermal layers.

Significance to Biomedical Research and the Program of the Institute:

Skin tumorigenesis is a widely used model to study chemical carcinogenesis and test the carcinogenic potency of compounds. The enzymes under study play an important role in the activation or detoxification of potential carcinogens. Their understanding is essential for the design and analysis of skin carcinogenesis systems.

Proposed Course of Project:

To determine the enzyme activity in various structures and cell types of skin. To study the metabolism of carcinogenic polycyclic hydrocarbons by the cutaneous enzymes. To determine the activity of cutaneous polycyclic hydrocarbon oxide hydrases.

Publications

Wiebel, F.J.: Cutaneous carcinogenesis: metabolic interaction of chemical carcinogens with skin. In Lee, D.H.K and Murphy, S.D. (Eds.): Environmental Physiology (in press).

Wiebel, F.J., Leutz, J.C. and Gelboin, H.V.: Aryl hydrocarbon (benzo(a)pyrene) hydroxylase: a mixed-function oxygenase in mouse skin. J. Invest. Dermatol. (in press).

Project No. Z01-CP-04592-01-CH
1. Chemistry Branch
Carcinogenesis, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Metabolism of Benzopyrene in Human Liver and Lymphocytes

Previous Serial Number: None

Principal Investigator: James K. Selkirk, Ph.D.

Other Investigators: Harry V. Gelboin, Ph.D. and James P. Whitlock, M.D.

Cooperating Units: None

Man Years:

Total	:	0.4
Professional	:	0.2
Other	:	0.4

Project Description

Objectives:

To determine how human tissue metabolize benzopyrene and to compare it to metabolic patterns in rodent systems.

Methods Employed:

Cell culture, high pressure liquid chromatography.

Major Findings:

It has been determined that human liver and lymphocytes produce several new derivatives not found in rodent liver.

Significance to Biomedical Research and the Program of the Institute:

It is of prime importance to know human metabolism of carcinogenic polycyclic hydrocarbons. Benzo(a)pyrene is a major environmental pollutant and may have a significant role in human cancer.

Proposed Course of Project:

To identify and determine the carcinogenity of these new derivatives.

Project No. Z01-CP-04779-05-CH
1. Chemistry Branch
Carcinogenesis, DCCP
2. Molecular Carcinogenesis
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Evaluation of Various Compounds for Their Effects as Inducers and Inhibitors of Aryl Hydrocarbon Hydroxylase Activity and Tumorigenesis

Previous Serial Number: Same

Principal Investigator: Friedrich J. Wiebel, M.D.

Other Investigators: Harry V. Gelboin, Ph.D. and James K. Selkirk, Ph.D.

Cooperating Units: None

Man Years:

Total	:	1.4
Professional	:	0.6
Other	:	0.8

Project Description

Objectives:

To develop methods to determine the activity of various compounds as inducers or inhibitors of carcinogen hydroxylating enzymes and polycyclic hydrocarbon tumorigenesis.

Methods Employed:

Cell culture techniques, spectrophotofluorometry, recording spectrophotometry, fluorescent microscopy and radioisotope studies.

Major Findings:

Butylated hydroxytoluene (BHT) and butylated hydroxy-anisole (BHA), two antioxidants commonly used as food preservatives, have been investigated as modulators of mixed-function oxygenase activity involved in polycyclic hydrocarbon metabolism. Both compounds are strong inhibitors of the constitutive benzo-(a)pyrene (BP) hydroxylase of rat liver in vitro. The antioxidants reduce the formation of BP-phenols, -quinones, and -dihydrodiols to a similar degree

indicating an inhibitory effect on the initial BP-oxide formation by the microsomal oxygenases. Inhibition of BP-metabolism by the antioxidants is paralleled by inhibition of microsome-dependent binding of BP-H^3 to DNA. Previous results indicated that the antioxidants might inhibit polycyclic hydrocarbon tumorigenesis by the induction of carcinogen metabolizing enzymes in liver, i.e. increased detoxification. The present data suggests that they may also reduce carcinogen activation by constitutive oxygenases.

Significance to Biomedical Research and the Program of the Institute:

Compounds of the type investigated can be used to explore the mechanism of enzymatic activation of carcinogenic polycyclic hydrocarbons and of tumor initiation by chemical carcinogens. It also is important to understand the influence of these food additives on the enzyme systems metabolizing xenobiotics and consequently on their biological effects.

Proposed Course of Project:

To use antioxidants such as BHA or BHT to explore the activation mechanism of carcinogenic polycyclic hydrocarbons in extrahepatic tissues *in vitro* and *in vivo*, to alter the course of tumorigenesis.

Publications

Wiebel, F.J. and Gelboin, H.V.: Aryl hydrocarbon (benzo(a)pyrene) hydroxylases in liver from rats of different age, sex and nutritional status: Distinction of two types by 7,8-benzoflavone. Biochem. Pharmacol. (in press).

Wiebel, F.J. and Gelboin, H.V.: Enzyme induction and polycyclic hydrocarbon metabolism in cell culture, experimental animals and man. In Montesano, R. and Tomatis, L. (Eds): Chemical Carcinogenesis Essays. IARC, Scientific Publications No. 10 Lyon, International Agency for Research on Cancer, 1974, pp. 57-82.

Wiebel, F.J., Whitlock, J.P., and Gelboin, H.V.: Mammalian aryl hydrocarbon hydroxylases: Mechanism of induction and role in carcinogenesis. In Khan, M.A.Q. and Bederka, J.P., Jr. (Eds): Survival in Toxic Environments. Academic Press, 1974, pp. 261-293.

Project No. Z01-CP-04537-01-CH
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on the Control of Initiation of DNA Synthesis

Previous Serial Number: None

Principal Investigator: Tsuyoshi Kakefuda, M.D., Ph.D.

Other Investigators: J. Tomizawa*, Ph.D. and Tina M. Bak

Cooperating Units: *Laboratory of Molecular Biology, NIAMDD, NIH, Bethesda, Maryland

Man Years:

Total	1.50
Professional	1.00
Other	0.50

Project Description

Objectives:

To observe the biochemical and genetic factors necessary for the initiation of DNA synthesis by establishing defined in vitro DNA-synthesizing model systems.

Methods Employed:

(A) Plasmid DNA system: Extracts of E. coli YS10 (Col E1) and YS1 (Col E1) were prepared from freeze-thawed bacteria by subsequent treatment with lysosyme and 5% Brij 58, followed by centrifugation at 25,000 rpm (SW50.1) for 20 minutes. The supernatant was passed through a Sephadex G25 column. Non-labeled circular, supercoiled, Colicin E1 DNA was prepared by CsCl-ethidium bromide density gradient centrifugation. DNA synthesis in vitro was assayed by incubating the purified DNA with a media containing cell extract, rNTP's, dNTP's, NAD, $MgCl_2$, KCL and phosphate buffer. The nascent DNA was labeled with either ^{32}P-dTTP or ^{32}P-dATP and BudR and was analyzed by centrifugation in either CsCl or sucrose gradients. The initiation and direction of synthesis were determined by treating DNA with R1 restriction enzyme (only a single sensitive site exists on Col E1 DNA). Molecules with replication loops were observed under an electron microscope.

(B) Mitochondria DNA System: Mitochondria were isolated from HeLa cells which were labeled with ^{14}C-TdR for 24 hours. The reaction mixture and product analysis were similar to that of Plasmid DNA except Brij 58 was replaced with NP 40 and nascent DNA was labeled with ^{3}H-TTP.

Major Findings:

Incorporation of dTMP into Col E1 DNA was dependent on incubation time, the presence of other dNTP's as well as the four rNTP's, and was completely inhibited by rifampicin. Synthesis initiated at a site 17 to 20% of the molecular length from the R1 restriction enzyme sensitive site and proceeded unidirectionally. This *in vitro* system is capable of complete semi-conservative replication and is no different from replication *in vivo* in terms of the initiation site, direction of replication and the final DNA product. Spermidine and glycerol enhanced initiation but blocked elongation of the deoxypolynucleotide chain. No new protein synthesis was required for initiation. The results indicated that initiation of DNA synthesis is controlled by the synthesis of a primer ribonucleotide sequence at a specific site on the DNA molecule.

In the case of mitochondrial DNA synthesis *in vitro*, incorporation of dTMP was dependent on other dNTP's but not on exogenous rNTP's in the assay mixture. The question of whether or not new RNA synthesis is required or whether the ribonucleotide pool in mitochondria is sufficient to synthesize RNA primer is under study. Oligomeric DNA molecules began replication at one site for each unit size of DNA present in the case of Plasmid DNA.

Significance to Biochemical Research and the Program of the Institute:

One typical cancer phenotype is enhanced or uncontrolled synthesis of DNA. The synthesis of DNA once initiated, replication usually continues to the end even under strong inhibitory conditions. The initiation of DNA synthesis, on the other hand, appears to be determined by very specific and sensitive elements. Biochemical and genetic information regarding the mechanisms involved in the initiation of DNA synthesis is extremely important for our understanding of normal and malignant cell reproduction.

Proposed Course of Project:

Our recent experiments indicated that RNA primer can be synthesized by RNA polymerase and rNTP's without the addition of cell extracts. The DNA coupled with RNA primer thus formed is capable of initiating DNA synthesis when it is transferred to complete media containing cell extract and rifampicin. The systems are to be further refined and simplified for precise analysis of factors necessary for initiation and elongation of the deoxypolynucleotide chain. Effect of various drugs including carcinogens, mutagens, alkylating agents and radiation will be studied.

Publications

Tomizawa, J., Sakakibara, Y., and Kakefuda, T.: Replication of colicin E1 plasmid DNA extracts. Origin and direction of replication. Proc. Nat. Acad. Sci. USA 71: 2260-2264, 1974.

Tomizawa, J., Sakakibara, Y., and Kakefuda, T.: Replication of colicin E1 plasmid DNA added to cell extracts. Proc. Nat. Acad. Sci. USA (in press).

Sugino, Y., Tomizawa, J., and Kakefuda, T.: The location of non-DNA components of closed circular colicin E1 plasmid DNA. Nature 253: 652-654, 1975.

Project No. Z01-CP-04554-01-CH
1. Chemistry Branch, Carcinogenesis, DCCP
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: In Vitro Transformation of Human Cells by Chemical Carcinogens

Previous Serial Number: None

Principal Investigator: Takeo Kakunaga, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	0.4
Professional	:	0.4
Other	:	0.0

Project Description

Objectives:

To establish conditions and methods for a quantitative assay of in vitro chemical transformation of human cells.

Methods Employed:

The procedures performed are based primarily on the information obtained with the development of quantitative assays for in vitro chemical transformation of animal cells, such as hamster and mouse cells. The experiences of the principal investigator with the development of a system for malignant transformation of hamster embryonic cells and A31-714 cells, a clone derived from BALB/3T3 mouse cell line, have been helpful.

The cells used have been freshly isolated from human embryo and from other normal human cell lines. Cell strains established from biopsies taken from persons who have a condition known to predispose them to malignancies, such as xeroderma pigmentosum and polyposis of the colon are also used. The cells are grown as a mass culture or as individual cells and then exposed to chemical carcinogens.

Morphology of the cells is examined by microscopy before or after fixation and staining. Cells which show altered morphology or growth patterns are picked

up and tested for their ability to grow in soft agar and for tumorigenicity in nude mice. Various experimental factors including choice of serum, concentration of serum, cell density, and number of allowed cell divisions after treatment are also examined.

Major Findings:

Fibroblasts (derived from biopsies taken from normal adult skin) in Eagle's minimum essential medium containing 10% fetal bovine serum have been successfully mass cultured. Using a selected lot of fetal bovine serum and untreated cells, cloning efficiencies as high as 80% have been regularly observed.

The susceptibility to the cytotoxic effects of chemical carcinogens was slightly higher in these cells than in A31-714 cells exposed to the same chemicals. Cells treated with various concentration of 4-nitroquinoline-1-oxide (4NQO) or N-methyl-N'-nitro-N-nitrosoquanidine (MNNG) for various times, and incubated for 4 to 6 weeks in the medium with various kinds and concentrations of serum but without transfer of cultures did not show any morphological changes. These cells also did not show any ability to grow in soft agar when tested 2 or 6 weeks after treatment.

However, cells treated with 4NQO for 30 min in suspension and cultured with transfer of cultures at a 1:4 dilution as soon as they reached confluence did show morphological alteration such as higher cell density, loss of contact inhibition, and slight crisscross pattern of cell growth, 4 or 5 weeks after treatment. These morphologically altered cells had an ability to grow in soft agar.

This morphological transformation has been also seen in cultures treated with MNNG. No cell growth alterations were seen in any of the control cultures.

Significance to Biomedical Research and the Program of the Institute:

To date there has been no reproducible system established in which human cells are transformed *in vitro* by chemicals. Rationale for the establishment of such a system is as sound as any at this time. If this project is successful, it will lead the way to many experiments in chemical and physical carcinogenesis which at present can only be carried out with animal cells. It would also provide a system in which to assay potential carcinogens directly on human cells, thus removing the necessity of extrapolating from data obtained from animal cells.

Proposed Course of Project:

To pursue the goals as outlined above in Objectives and to publish any results so obtained.

Project No. Z01-CP-04555-01-CH
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Factors Affecting Malignant Transformation by Chemical Carcinogens in Mouse Cells

Previous Serial Number: None

Principal Investigator: Takeo Kakunaga, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	0.4
Professional	:	0.4
Other	:	0.0

Project Description

Objectives:

To learn more about the mechanisms involved in the malignant transformation of cells in vitro, and to find the optimum conditions for the in vitro assay of chemical carcinogens.

Methods Employed:

A quantitative system for the assay of malignant transformation of A31-714 cells by chemical carcinogens which was developed previously (Int. J. Cancer 12: 463-473, 1973) is used. Culture conditions and genetic and physiological states affecting the transformation frequency are identified. These include the kinds and concentrations of serum, the growing state of cells, cell density, phase of cell cycle, and the isolation and use of mutants cell lines which show different susceptibility to chemical carcinogens. Compounds which inhibit the synthesis of macromolecules, excision repair of DNA, or postreplication repair of DNA, will be used to examine their effects on the frequency of transformation by 4-nitroquinoline-1-oxide (4NQO).

Major Findings:

Transformation frequency by chemical carcinogens was generally decreased in medium containing fetal bovine serum compared to that in the medium containing new born calf serum.

Experiments using hydroxyurea, a reversible inhibitor of semiconservative DNA synthesis, and the property of density dependent inhibition of cell growth, indicated the following: 1) Several cell divisions were required for the appearance of the transformed cells after treatment with chemical carcinogens. Cells maintained in a non-growing state after carcinogen treatment showed a markedly reduced transformation rate. 2) One cell generation was required for the fixation of transformation, and more than three cell generations were required for the expression of the transformed state.

Caffeine, which has been reported to inhibit postreplication repair but not excision repair of DNA in animal cells, reduced the transformation frequency when added to the culture medium during the first S phase after 4NQO treatment. The presence of caffeine in the medium at other times had no effect on the 4NQO induced transformation.

Acriflavin was found to be reversible inhibitor of unscheduled DNA synthesis. Exposure of the cells to this compound for 24 hrs starting immediately after 4NQO treatment resulted in an increase in the cell transformation frequency.

Significance to Biomedical Research and the Program of the Institute:

The results with this project will provide: 1) Optimum conditions needed for the assay of potential chemical carcinogens using the A31-714 cell system; 2) Basic information needed to develop assay systems using human cell lines. Thus, this project is directly aimed at that aspect of the Program of the Institute which seeks to find rapid, meaningful assays for potential chemical carcinogens.

Proposed Course of Project:

To pursue the goals outlined above in Objectives and to publish any results so obtained.

Publications

Kakunaga, T.: Requirement for cell replication in the fixation and expression of the transformed state in mouse cells treated with 4-nitroquinoline-1-oxide. Int. J. Cancer 14: 736-742, 1974.

Kakunaga, T.: The role of cell division in the malignant transformation of mouse cells treated with 3-methylcholanthrene. Cancer Res. (in press).

Project No. Z01-CP-04558-02-CH
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Role of DNA Repair Mechanisms in In Vitro Chemical Transformation

Previous Serial Number: Same

Principal Investigators: Takeo Kakunaga, Ph.D. and C. Wesley Dingman, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	0.6
Professional	:	0.6
Other	:	0.0

Project Description

Objectives:

To learn more about the mechanisms involved in the malignant transformation of cells. In particular, to develop an experimental system in which one can attempt to correlate the DNA repair efficiency of various cell strains with their propersity to undergo malignant transformation in vitro in response to various chemical and physical agents.

Methods Employed:

Currently efforts are being directed at finding appropriate techniques to assess DNA repair (particularly, DNA strand rejoining) in various established mouse cell lines which are known to differ significantly in the ease which they can be transformed with chemicals in vitro. Various ways of gently lysir cells, denaturing DNA, and measuring the size of the single-strands of DNA released by sedimentation in alkaline sucrose gradients are being tried. The most promising method so far has been the treatment of cells with anionic detergents at 50°C in the presence of a proteinase. This method is then applied to cells after various chemical treatments and after different post-treatment incubation times.

Major Findings:

Recent results indicate that strand breaking and rejoining in mouse cells can be observed in cells treated with 4-nitroquinoline-1-oxide. In order to observe this, single-stranded DNA molecules of the order of 450 x 10^6 daltons have now been reproducibly isolated and their sedimentation profile determined. Studies on the effects of caffeine, hydroxyurea and acriflavin on the rejoining process are underway.

Significance to Biomedical Research and the Program of the Institute:

The development of reliable techniques to measure the DNA repair efficiency of various cell lines will be very useful in assessing the degree of risk which humans of different genotypes have to permanent or severe DNA damage by various agents. Furthermore knowledge of the relationship (if any) between a cells ability to repair different types of DNA damage and the probability of malignant transformation in that cell will help in understanding some of the molecular mechanisms underlying carcinogenesis.

Proposed Course of Project:

To pursue the goals as outlined in Objectives and to publish any results so obtained.

Project No. Z01-CP-04785-05-CH
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Role of DNA Repair Mechanisms in the Etiology of Cancer

Previous Serial Number: Same

Principal Investigators: Rufus S. Day, III, Ph.D. and C. Wesley Dingman, M.D.

Other Investigators: Alfred Giuffrida, B.S.

Cooperating Units: None

Man Years:

Total	:	2.4
Professional	:	1.6
Other	:	0.8

Project Description

Objectives:

To learn more about DNA repair mechanisms in human cells and about their role in carcinogenesis. In particular, to determine the nature of the biochemical defects in human cell strains grown from biopsies taken from persons having extreme susceptibility to carcinogenesis, and to determine the biological consequences of the repair defects of these cells.

Methods Employed:

Using a previously developed Adenovirus 2 host cell reactivation assay, it has been possible to quantitate the deleterious effects of ultraviolet light (UV), nitrous acid, and psoralen plus near-UV light on the ability of the virus to initiate and sustain infection. The method involves establishing a monolayer fibroblast culture which is then infected with adenovirus which has been treated or not with the various agents listed above. The infected cells are then incubated 17-20 days with feeding by means of periodic overlaying with a nutrient agar. Non-treated virions or those treated ones "reactivated" by fibroblast cell repair mechanisms from countable plaques of dead, lysed cells. With this method the plating efficiency of unirradiated adenovirus is the same on all of the non-transformed human cell strains so far tested. It is 5 to 100 fold greater when human tumor lines of various types are used.

Major Findings:

(1) Virus damaged by ultraviolet light: (a) Five strains of xeroderma pigmentosum (XP) fibroblasts have been collected, which, by the repair assays of others, have shown a normal ability to repair UV-damaged DNA. Such fibroblasts are termed "XP variant". All five of these strains have a partial defect in the repair of UV damaged adenovirus of a similar magnitude (~40%). This result establishes a new and now definable class of XP patients, and the adenovirus test can be used in an aid to clinical diagnosis of the condition. (b) The classical (or non-XP variant) XP condition can arise from alterations in any one of five different genetic loci definable by complementation analysis (see annual report of Dr. J. H. Robbins, Dermatology Branch, NCI). In collaboration with Drs. J. H. Robbins and K. H. Kraemer, it has been shown that xeroderma cells from two different genetic origins (complementation groups) can (when fused together to form heterokaryons) repair UV damage to irradiated adenovirus, demonstrating for the first time that such fused cells are capable of biologically meaningful repair.

(2) Virus damaged by nitrous acid: In this laboratory it has been found that normal human fibroblasts can repair nitrous acid-damaged adenovirus whereas XP fibroblasts are deficient in such repair. Since UV is presumably the agent causing skin cancer in XP patients, and since XP cells are deficient in the repair of UV-damaged DNA as well as DNA damage caused by other known carcinogens, it may well be that nitrous acid, which by itself makes repairable damage in DNA and which combines with amines to form nitrosamine (a known carcinogen), should be viewed as a potential human carcinogen.

(3) Virus damaged by DNA cross-linking agents: It has recently been reported that cells from persons afflicted by Fanconi's anemia are more easily damaged than normal cells by DNA crosslinking agents such as mitomycin C, nitrogen mustard, and psoralen plus near-UV (320-390 nm) treatment. Although it was established that psoralen plus near-UV light treated virus is less repaired in XP cells than in normal cells, no difference between the ability of Fanconi's anemia cells and normal cells to repair similarly treated virus was found. By measuring the number of psoralen plus near-UV produced DNA crosslinks in the viral DNA (using electrophoresis in polyacrylamide-agarose gels and sedimentation in alkaline sucrose density gradients), it was found that even normal cells are probably unable to repair crosslinks in the viral DNA. Therefore, even if Fanconi's cells are unable to repair DNA crosslinks in their own (cellular) DNA, the use of the virus assay to detect repair of crosslinks in viral DNA cannot be used to observe such a defect.

Significance to Biomedical Research and the Program of the Institute:

Physical, chemical, and viral carcinogens are all known to alter the structural integrity of the cellular genetic apparatus. An evaluation of the role of DNA repair and/or related mechanisms in conferring resistance or susceptibility to mutagenesis and carcinogenesis is an important facet in any overall program having as its goal the understanding of the molecular pathways which, when perturbed, give rise to carcinogenesis in humans. It is a long range goal of

this project to determine whether or not the elucidation of genetic repair mechanisms is important to the understanding of carcinogenesis, and it is expected that an understanding of genetic repair mechanisms in general will benefit many areas of biomedical research.

Proposed Course of Project:

To pursue the goals outlined above in Objectives and to publish these results.

Publications

Day, Rufus S., III.: Studies on repair of adenovirus 2 by human fibroblasts using normal, xeroderma pigmentosum, and xeroderma pigmentosum heterozygous strains. Cancer Res. 34: 1965-1970, 1974.

Day, Rufus S., III.: Human cells repair DNA damaged by nitrous acid. Mutation Res. (in press).

Day, Rufus S., III.: Xeroderma pigmentosum variants have decreased repair of UV-damaged DNA. Nature (in press).

Day, R. S., III, Kraemer, K. H., and Robbins, Jay H.: Complementing xeroderma pigmentosum fibroblasts restore biological activity to UV-damaged DNA. Mutation Res. (in press).

Day, Rufus S., III.: The use of human adenovirus 2 in the study of the xeroderma DNA repair defect. In Hanawalt, P. C. and Setlow, R. B. (Eds.): Molecular Mechanisms for Repair of DNA. New York, Plenum Press, (in press).

Project No. 701-CP-04787-05-CH

1. Chemistry Branch, Carcinogenesis, DCCP
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mode of Replication of RNA Tumor Viruses

Previous Serial Number: Same

Principal Investigator: Tsuyoshi Kakefuda, M.D., Ph.D.

Other Investigators: Tina M. Bak

Cooperating Units: None

Man Years:

Total	1.00
Professional	0.50
Other	0.50

Project Description

Objectives:

To observe the early and late events of viral RNA replication and the integration of virus-directed DNA into host genome after infection with RNA tumor virus.

Methods Employed:

Chicken embryo cells were infected with Schmidt-Ruppin strain of Rous sarcoma virus. Virus specific DNA was selectively isolated from the cytoplasm of newly infected cells by using molecular hybridization techniques as previously described. Cellular DNA from RSV transformed chick cells was also extracted and sheared by vigorous pipetting. SR-C3H-2127 mouse cells in which the RSV genome is integrated but not fully expressed were treated with DEAE-dextran for a short time and then incubated in culture with the DNA described above. The DNA is either untreated or treated with DNAase, RNAase, RNAase H, sonicated, heated, or alkalie treated before adding it to the cell culture. The control cells were either not challenged by DNA or omitted from the DEAE-dextran treatment.

Incorporation of intact viral DNA into host cell DNA was studied by: 1) the production of infectious virus; and 2) experiments in which density labeled exogenous viral DNA was incubated with cells after which cellular DNA was isolated and banded in CsCl or sedimented in alkaline sucrose gradients.

Major Findings:

Viral RNA-directed DNA was found to be synthesized within 3 hours in the cytoplasm (membrane fraction) as indicated by earlier studies (Kakefuda et al. Cancer Res. 34: 679-688, 1974). The DNA is comparable with viral RNA in size, is hybridizable to viral RNA, and may be a complete copy of viral RNA. No such DNA was synthesized in uninfected control cells. The biological activity of the nascent viral DNA is now being studied.

Significance to Biomedical Research and the Program of the Institute:

The transcription of viral RNA to DNA and the incorporation of the latter into the genetic system of the host cell are important steps in viral carcinogenesis. Evidence is now being accumulated indicating that virus-directed DNA is synthesized in the cytoplasm a short time after infection and integrated in the host chromosome by translocation to the nucleus. It is important to elucidate these mechanisms to understand the molecular events occurring during viral carcinogenesis. Such information would be highly useful in designing methods to prevent or control malignancies of viral origin.

Proposed Course of Project:

There are a few other mammalian cells containing the RSV genome in masked or defective form (e.g. R-R 1022 and HT-1). Experiments using these systems will be carried out. Bromodeoxyuridine and other compounds which are known to induce the viral genome and enhance gene translocation will be applied to these systems.

Publications

Kakefuda, T.: Electron microscopic observations on cells of the blood and bone marrow. In Custer, R. P. (Ed.): An Atlas of Blood and Bone Marrow. Philadelphia, London and Toronto, W. B. Saunders, 1974, pp 441-521.

Kakefuda, T., Dingman, C.W., Bak, T.M., Hatanaka, M., and Kitano, Y.: Reverse transcription of the viral genome associated with the plasma membrane after infection with RNA tumor viruses. Cancer Res. 34: 679-688, 1974.

Project No. Z01-CP-04525-03-CH
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Electrophoretic Techniques for Protein, RNA, and DNA

Previous Serial Number: Same

Principal Investigator: Andrew C. Peacock, Ph.D.

Other Investigators: Sylvia L. Bunting, M.S. and Roswell A. Reed

Cooperating Units: None

Man Years:

Total	2.8
Professional	2.8
Other	0.0

Project Description

Objectives:

To investigate the influence of primary structure and conformation on the electrophoretic and biological properties of proteins and nucleic acids and to use this information in devising improved methodology for analysis and purification.

Methods Employed:

Cultures of HeLa cells, assay of radioisotopes, isolation and electrophoresis of RNA and DNA, optical scanning of stained and unstained gels, preparative electrophoresis.

Major Findings:

1. Studies of conformational isomerism of ribosomal RNA (rRNA). The property of at least three conformational isomers of 18S rRNA to co-exist in solution was further studied. The two most rapidly migrating isomers were easily interconvertible, but conditions for producing only the third, more slowly migrating form could not be found. This isomer could, however, be produced in about 50% yield from either of the faster forms. Double-labeled RNAs were used to show that, after conversion to one or another discrete isomer, all forms could be converted by dimethyl sulfoxide to the same random coil inter-

mediate. From this, mixed forms of the original isomers could be regenerated. This experiment was taken as proof that all forms share the same configuration when denatured and that all the multiple forms of 18S RNA can be derived from the same molecule.

2. Studies on conformational properties of nucleic acids. In contrast to the previous studies in which the samples were treated in various ways and evaluated under standard electrophoretic conditions, a lengthy series of experiments was performed in which the only variable was the electrophoretic conditions. The variables were ionic strength ([Na] 1 to 100 mM) and temperature (t=0-90°). Many conformational transitions have been observed by spectroscopic methods in this region. By electrophoresis, however, we detected only one phenomenon, namely, the extension of the molecules, probably due to phosphate repulsion at lower salt concentrations. This property was freely reversible and resulted in a progressive reduction in electrophoretic mobility. The small transfer RNA (tRNA) molecules behaved as if they were much larger under these conditions. The sodium concentration for 50% extended form was a little higher at 90° than at 0°. All classes of nucleic acids (including DNA, nuclear RNA, etc.) showed this property. Magnesium ions in the range 10^{-4} to 10^{-3} M prevented or reversed this extension.

The electrophoretic property was most closely correlated with the intrinsic viscosity of the nucleic acid. Increases in electrophoretic mobility with temperature were mainly ascribable to decreases in the viscosity of the buffers.

Initiator tRNA behaved differently. Above 25° it migrated significantly faster than other tRNAs. The possibility that this resulted from cleavage of the molecule was ruled out by showing that the change was reversible. The significance of a unique conformation for this functionally unique molecule will be explored further.

3. Preparative electrophoresis. Preparative electrophoresis of RNA has been under active study. The recovery of RNA from the gels as intact molecules has so far not been generally successful. Because several steps are involved in elution from gel separation from impurities arising from the gel, concentration and reanalysis, many possibilities have been examined in an effort to find the most suitable combination of procedures. Random contamination by ribonucleases has frequently been a source of confusion.

Elution has been achieved by (1) homogenization of gel, (2) electrophoretic elution into buffers of special composition to retain the eluted RNA, and (3) electrophoretic elution with eluant retained by a dialysis membrane. It is not now known which of these is preferable.

Significance to Biomedical Research and the Program of the Institute:

The ability to conduct detailed studies on interrelationships between macromolecules, such as RNA, proteins, and DNA, is probably fundamental to under-

standing the alterations which occur in malignancy. The above studies are directed towards this goal.

Proposed Course of Project:

Most of the studies described above are currently incomplete, and will be continued. The mechanisms by which RNA synthesis is controlled will be studied by a comparison between experimental results and computer simulation of proposed models.

Publications

Zeiger, R. S., Salomon, R., Dingman, C. W., and Peacock, A. C.: Role of base composition in the electrophoresis of heat-treated deoxyribonucleic acid from HeLa and mouse cells in composite polyacrylamide gels. Biochem. 13: 3388-3393, 1974.

Pastewka, J. V., Ness, A. T., and Peacock, A. C.: Hemoglobin binding by isolated polymeric proteins from human haptoglobin types 2-1 and 2-2. Biochim. Biophys. Acta (in press).

Project No. Z01-CP-04562-02-CH
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Properties of Cytoplasmic RNAs in Mammalian Cells

Previous Serial Number: Same

Principal Investigator: Keiji Nishinaga, Ph.D.

Other Investigators: Andrew C. Peacock, Ph.D. and Sylvia L. Bunting, M.S.

Cooperating Units: None

Man Years:

Total	1.2
Professional	1.2
Other	0.0

Project Description

Objectives:

To devise analytical approaches for studies of conformational changes of ribosomal RNAs and to use these techniques to get an understanding of control of processing of RNA in mammalian cells.

Methods Employed:

Culture of HeLa cells, assay by radioisotopes and sucrose gradient centrifugation, electrophoresis and optical scanning of stained gels.

Major Findings:

Analysis of 18S RNA in rat liver, mouse liver, and HeLa cells by electrophoresis. Electrophoresis of 18S RNA in 2.2% or 2.5% acrylamide-0.5% agarose gels gave some indication that 18S RNA isolated by a cold extraction has at least two bands whereas these same RNAs isolated with the hot extraction show another one band. The electrophoresis of 18S RNA isolated by cold phenol extraction showed three bands. One of these three bands is converted to one band after heat-treated and slowly cooled in the presence of different concentration of Mg^{2+} ion. With labeled 18S RNA similar observations were made on RNA extracted with hot phenol.

Significance to Biomedical Research and the Program of the Institute:

The ability to conduct detailed studies on interrelationships between RNA and DNA is probably fundamental to understanding the control mechanism which differentiates between normal and malignancy. The above studies are directed towards this goal.

Proposed Course of Project:

Most of the studies described above are currently incomplete and will be continued.

Project No. Z01-CP-04563-02-CH
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of a Cationic Carbocyanine Dye

Previous Serial Number: Same

Principal Investigator: Marie R. Green, Ph.D.

Other Investigators: Jullia V. Pastewka

Cooperating Units: None

Man Years:

Total	:	1.0
Professional	:	1.0
Other	:	0.0

Project Description

Objectives:

To obtain more information about the characteristics of the dye 1-ethyl-2-[3-(1-ethylnaphtho[1,2d]thiazolin-2-ylidene)-2-methylpropenyl]naphtho[1,2d]-thiazolium bromide and its interaction with macromolecules. Identification of acidic macromolecules on gels and tissue sections by metachromatic reactions.

Methods Employed:

Polyacrylamide gel electrophoresis, staining and scanning of gels with a Gilford spectrophotometer, absorption measurements of dye solutions in a Cary recording spectrophotometer, observation of color reactions of pure substances in films and solutions, enzymatic digestions and solvent extractions of cryostat sections, microscopic observation of stained sections.

Major Findings:

The simultaneous differential staining of phosphoproteins, proteoglycans, sialoglycoproteins, proteins and nucleic acids in deparaffinized tissue sections has been previously demonstrated. The same reactions were shown to occur in frozen sections. An additional class of substances, the polar lipids, was demonstrated in cryostat sections using this stain under specified conditions of fixation and mounting. Polar lipids were demonstrated in

mitochondria, nerve, brain, retina, and on inner and outer surfaces of tissues.

The staining of several representative glycoproteins electrophoresed on polyacrylamide gels was investigated using this dye. It was found that sialic acid-rich glycoproteins stained blue-green and could be distinguished from other proteins which stained red. After neuraminidase treatment to the desialylated form, these glycoproteins stained red-purple.

Significance to Biomedical Research and the Program of the Institute:

This newly developed histochemical staining procedure is simple and specifies classes of macromolecules by color. It should find wide application as a method for the identification of secretions and cellular inclusions in pathologic diagnosis and in research investigations.

The identification of minute amounts of sialic acid-rich glycoproteins on gels is very useful for identifying unknown substances in a mixture of proteins when amounts available are too small for other chemical analyses. This procedure identifies these substances without radioactive labeling.

Sialic acid-containing glycoproteins are constituents of membranes and play an important role in recognition phenomena. They occur in HL-A antigens, M and N blood groups, CEA antigens, etc. This procedure may be useful in identifying some of these substances.

Proposed Course of Project:

The histochemical method will be applied for diagnosis of biopsy and cytology materials. A study of rodent and human breast tissue is in progress.

The method for identifying phosphoproteins and sialic acid-rich glycoproteins with this dye is being used for the identification of these proteins in milk and breast tissue. Studies of antigens in tissues and sera are planned.

Publications

Green, M. R. and Pastewka, J. V.: Simultaneous differential staining by a cationic carbocyanine dye of nucleic acids, proteins and conjugated proteins. I. Phosphoproteins. J. Histochem. Cytochem. 22: 767-773, 1974.

Green, M. R. and Pastewka, J. V.: Simultaneous differential staining by a cationic carbocyanine dye of nucleic acids, proteins and conjugated proteins. II. Carbohydrate and sulfated carbohydrate-containing proteins. J. Histochem. Cytochem. 22: 774-781, 1974.

Green, M. R.: Simultaneous differential staining of nucleic acids, proteins, conjugated proteins and polar lipids by a cationic, carbocyanine dye. J. Histochem. Cytochem. (in press).

Project No. Z01-CP-04782-05-CH
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Hormonal Effects on Normal and Malignant Breast Tissue in Culture

Previous Serial Number: Same

Principal Investigator: Marie R. Green, Ph.D.

Other Investigators: Jullia V. Pastewka and Andrew C. Peacock, Ph.D.

Cooperating Units: None

Man Years:

Total	:	1.0
Professional	:	1.0
Other	:	0.0

Project Description

Objectives:

To study the response of normal and malignant breast tissue in culture to hormones in terms of RNA and DNA synthesis and the production of proteins (particularly milk proteins). To gain a better understanding of hormone regulation of nucleic acid and protein synthesis of mammary tissue.

Methods Employed:

Culture of mammary explants, isolation of RNA and DNA, separation on agarose-acrylamide gels, radioactive tracer techniques, isolation and purification of proteins from explants and milk, polyacrylamide gel electrophoresis, examination of tissue sections by histochemical methods, autoradiography.

Major Findings:

Milk proteins from BALB/c and C3H mice were characterized with respect to their electrophoretic migration in polyacrylamide gels under alkaline and acid conditions. Phosphoproteins and sialic acid-rich glycoproteins were identified by staining with "Stains-all" and by the changes in electrophoretic mobility and staining induced by prior treatment with phosphatase and neuraminidase, respectively. Glycoproteins were identified by staining with periodic acid schiff. The two major whey proteins were further characterized

by their migration in SDS gels. One protein had the same mobility as mouse serum albumin; the other protein migrated with a mobility similar to that of bovine and human α-lactalbumin. The major casein and whey proteins from each strain migrated similarly under acidic and basic electrophoretic conditions.

This analysis has permitted the identification of proteins present normally in virgin and pregnant mammary tissues of mice. Analysis of proteins in mammary tumors is in progress. The synthesis and degradation of these proteins in mammary explants as regulated by hormones in vitro is in progress.

Significance to Biomedical Research and the Program of the Institute:

The mouse has long served as a model for the study of mammary tumors. It has been demonstrated that the C3H strain carries the mouse mammary tumor virus, MTV. A high incidence of tumors may be produced in BALB/c mice, which normally have a low incidence of mammary tumors, by foster-nursing the pups on C3H, MTV+ mice. The mechanism by which the presence of the virus induces the emergence of the mammary tumors is not known. It is known that environmental and hormonal factors influence the course of tumor development. Recently, the viral proteins have been characterized. The analysis of milk proteins will permit the study of changes which occur during the carcinogenic process and perhaps the identification of products characteristic of the tumor in serum. It is possible also that early changes in preneoplastic tissue may be detected. Such findings can then be applied to studies of biopsy material from humans.

Proposed Course of Project:

The study of the mechanism of hormone regulation in normal and malignant mammary tissue will continue.

Publications

Green, M. R. and Pastewka, J. V.: Identification of sialic acid-rich glycoproteins on polyacrylamide gels. Anal. Biochem. (in press).

SUMMARY REPORT

EXPERIMENTAL PATHOLOGY BRANCH

July 1, 1974 through June 30, 1975

"Plans, develops, and implements a coordinated research program involving (1) development and characterization of experimental models of major forms of human cancer; (2) development of techniques for experimental study of the etiology and pathogenesis of cancer in human tissues; (3) analysis of stages in pathogenesis of human cancer and their suitability for the development of preventive measures; (4) biochemical studies on the metabolism of chemical carcinogens in selected animal tissues; (5) development of standards of pathology for the evaluation of carcinogenic effects; and (6) planning and conducting research on the etiology and pathogenesis of cancer of the human digestive system."

The Branch includes the Office of the Chief, three sections (In Vitro Pathogenesis, Perinatal Carcinogenesis, Tumor Pathology), and an Ultrastructure Unit. During FY 74 a reorganization transferred the Tumor Pathology Section from the Office of the Associate Director to the Experimental Pathology Branch, while the former Endocrine Carcinogenesis Section transferred its activities to the Frederick Cancer Research Center and the former Digestive System Carcinogenesis Section was abolished and some of its activities transferred to the Office of the Branch Chief.

The Experimental Pathology Branch investigates the pathogenesis of neoplastic disease using morphological and biochemical approaches at all levels of biological organization, from human tissues and animal models to organ and cell cultures and molecular interactions.

Increasing emphasis is given to carcinogenesis studies using in vitro cell and organ culture systems, particularly with epithelial cells. Pure populations of mouse epidermal cells, hair follicle cells and dermal fibroblasts have been developed and used to study neoplastic transformation as well as mechanisms of initiation and promotion.

The role of cell differentiation in the neoplastic transformation of epithelia is also studied. In vitro alteration from keratinizing to mucosecretory differentiation of epidermal cells has been obtained with retinyl acetate, thus offering a new model for these studies in addition to respiratory epithelium.

Interaction of chemical carcinogens and cell macromolecules in epidermal cells was further studied. Differences in the binding of different carcinogens to heterochromatin and euchromatin are under study. Guanine is selectively inserted in the repair process of DNA damage caused by skin carcinogens following low carcinogen doses which allow for cell survival; at higher doses in which survival is minimal, all bases are replaced, suggesting that guanine repair may be more specific for repair after sublethal damage.

Transplacental carcinogenesis studies using 7,12-dimethylbenz[a]anthracene have produced tumors and striking cytologic alterations in the developing

nervous system of Sprague-Dawley rats. In vitro studies are underway to examine cerebellar cells, which are markedly depleted as a result of this treatment, and to search for evidence of transformation.

Additional studies of neurogenic tumors in mice have shown a consistent presence of S-100 protein, which is specific for nervous tissue, and efforts are underway to verify the origin of several controversial experimental tumors of presumed neurogenic origin. This approach will, hopefully, allow more critical evaluation of tumors which, until now, have been defined by relatively non-specific methods.

Lung tumors induced in genetically resistant mice were found to be immunologically cross-reactive with normal lung tissue of genetically susceptible mice and with embryonic lung of resistant strains. Efforts are now underway to unmask common antigens on cell surfaces and characterize their nature and possible relationship to known carcinoembryonic antigens.

Histogenesis and diagnostic standardization of experimental cancers and comparisons with human counterparts are major activities in the Branch. The Registry of Experimental Cancer maintains an international reference center of pathologic material.

As a part of the efforts towards definition and standardization of tumor classification, a first workshop was held to define controversial tumors and related lesions in rat livers; its success will lay the groundwork for future workshops.

Thus, the Branch is developing towards an integrated multidisciplinary approach to tumor pathogenesis studies. The staff of this Branch is also largely involved in the scientific development and monitoring of collaborative research, particularly for the Biological Models Segment and the Bioassay Operations Segment.

In Vitro Pathogenesis Section - *"(1) In collaboration with other Sections of the Branch, develops in vitro models for studying the pathogenesis of major forms of human cancer utilizing both human and animal models; (2) utilizes these culture systems in biochemical, cytologic, and biologic investigations to develop approaches to the prevention of cancer in humans, specifically by studying the stages in the pathogenesis of cancer to learn which are amenable to arresting or reversing the progression of the disease before it threatens life; and (3) develops procedures for evaluating the biological potentiality of lesions induced by chemical or physical carcinogens in human tissues in vitro."* This Section has directed its efforts toward developing in vitro model systems to study mechanisms of epithelial carcinogenesis, since the large majority of human cancers are derived from epithelial cells. A classic model for induction of squamous cancer, mouse epidermis, has been adapted for growth in cell culture in this laboratory and previously shown to maintain biological function in a reasonably normal manner. During the past year this system has been further characterized and used for carcinogenesis studies. The epidermal cells demonstrated a natural synchronous growth cycle. Primary cells can be frozen in a viable state and synchronous growth is maintained after thawing. These cells can metabolize and bind the carcinogen

7,12-dimethylbenz[a]anthracene (DMBA). A high aryl hydrocarbon hydroxylase (AHH) activity and high levels of DMBA binding to DNA are found after 2-3 days in culture; these functions are reduced 2-4 fold by 10 days. This reduction can be abolished by DMSO in the culture medium, which also prolongs the life-span of primary cultures for as long as 6 months.

Transformation studies have been performed on epidermal cells using several techniques. Treatment with DMBA or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) for brief periods in young primary cultures and subsequent treatment with the active tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) has led to the emergence of several rapidly growing cell lines (not yet tested for tumorigenicity).

Epidermal cells, obtained from newborn mice treated transplacentally by injecting the pregnant mother with urethane, deomonstrated stimulated DNA synthesis. In one experiment, such cells transformed when subsequently treated with TPA. The transformed cells gave undifferentiated epithelial tumors in newborn mice and produced a plasminogen activator when tested for fibrinolytic activity. Other experiments in progress include short term in vitro growth and treatment combined with grafting cells back to the syngenic host and promoting in vivo. In addition a malignant epidermal cell line, designated EC-1, derived from a chemically induced squamous cell carcinoma of mouse skin, is being studied for properties in vitro. This line forms multi-cell aggregates when grown over 1% agar. These aggregates may be useful for injecting cells into hosts for tumorigenic assay. Other lines will be treated for such aggregation.

A 5-10 fold stimulation of DNA synthesis occurs in epidermal cells exposed in vitro to the promoter TPA. This response is dose dependent, occurs at doses as low as 10^{-8}M and is similar to that seen in vivo thus allowing one to study promotion in vitro. This reponse does not occur in dermal fibroblasts. The enzyme ornithine decarboxylast (ODC), a key enzyme in polyamine biosynthesis, is stimulated 10-20 fold within 6 hours of TPA treatment and remains elevated for 24 hours. This enyzme has previously been shown to be involved in proliferative and differentiative functions of other cell types.

Differentiation and its effects on factors related to carcinogenesis have also been studied in the mouse epidermal system. Vitamin A has been reported to reduce tumorigenesis in a number of epithelial systems. Retinyl acetate (RA), a vitamin A ester, converts these cells from a keratinizing to a secretory epithelium. There is a marked increase (10x) in galactose and mannose incorporation in vitamin A treated cells. Binding of DMBA to DNA is reduced as is AHH activity after treatment with RA. DNA synthesis is also diminished but repair synthesis after damage with UV light or bromomethylbenzanthracene is not altered. Thus factors believed to be related to epidermal carcinogenesis are dependent on the state of differentiation in epidermis, and the mouse skin system parallels respiratoryand glandular epithelium in its response to agents affecting differentiation and carcinogenesis.

Investigations into the interaction of chemical carcinogens with cellular macromolecules have continued. Specificity in the reaction of carcinogenic polynuclear hydrocarbons with DNA is studied on chromosomes isolated from

Chinese hamster cells exposed to radioactively labeled carcinogens. Preliminary evidence suggests that chromosomal subunits composed of heterochromatic portions of the genome may bind more benzo[a]pyrene than euchromatic portions of the genome. However binding by DMBA is equal in both types.

The repair of DNA damage caused by skin carcinogens β-propiolactone (BPL) and MNNG was studied using mouse skin cell cultures and tritiated deoxyribonucleosides. For each chemical, low doses of guanine, is specifically inserted in the repair process. With higher doses, at which survival is minimal, all precursors are replaced. Thus specific guanine repair may be more relevant to cell survival after damage by MNNG and BPL. This type of repair was not detectable in human WI-38 cells treated with MNNG but was seen in both mouse and rat epidermal cells and dermal fibroblasts. The kinetics for guanine specific repair differ from repair in which all 4 bases are replaced suggesting that they are distinct processes. Studies are now in progress to determine if cytosine specific repair is also present in mouse epidermis. Prelimary evidence suggests that this is the case in the mouse but not in human WI-38 cells.

The role of DNA replication at the time of initiation of carcinogenesis is also being studied using the mouse skin painting model in vivo. The application of croton oil to mouse skin results in a marked stimulation of DNA synthesis 18 hours later. When a single application of croton oil was given at times ranging from 6 hours before until 12 hours after initiation with urethane or MNNG, the papilloma yield was increased 2-3 fold at all times. This suggests that cell proliferation several hours after the initiator is applied may be important in tumor initiation. Studies in cell culture failed to show any difference in repair of MNNG damage in proliferating and resting cells.

Perinatal Carcinogenesis Section - "(1) Investigates the induction of cancer in experimental animals before birth and during infancy, with the goal of identifying factors responsible for the initiation and growth of tumor of childhood and infancy in man; (2) utilizes techniques of chemical synthesis, biochemistry, histopathology, immunology, and endocrinology to identify carcinogens to which both individually and in combination the fetus and neonate are particularly vulnerable, and to identify host factors which qualitatively or quantitatively modify their biological effects; and (3) develops measures for prevention cancer in children or in later life as a response to conditions of high susceptibility to carcinogens during fetal or childhood." Investigations of the carcinogenic activity of α-hydroxylated derivatives of dimethylnitrosamine (DMN) have shown that methyl(acetoxymethyl)-nitrosamine (DMN-OAc) is selectively carcinogenic to the mucosa of the small intestine and intestine and colon following a single intraperitoneal injection given to weanling rats. The selectivity of this compound suggests a useful role for it in experimental carcinogenesis of the gastrointestinal tract. The effects of this compound on neonates and by transplacental exposure, as well as the distribution and patterns of metabolism of the methyl-^{14}C compound, are under study.

The polynuclear aromatic hydrocarbon carcinogen DMBA has been shown to be a potent transplacental carcinogen in the Sprague-Dawley rat, with major effects

on the central and peripheral nervous system. Although it induces no medulloblastomas or other tumors of neuronal origin, DMBA causes severe necrosis of cells of the external and internal granular layers of the developing cerebellum, resulting in permanent severe depletion of cells of the internal granular layer. To determine whether there is a population of potential medulloblastoma cells among the residual granule cells in rats subjected to transplacental DMBA, a study is being initiated to cultivate these cells in vitro and to examine the resulting cultures for properties of transformed cells.

In collaboration with Prof. Wolfgang Wechsler of the Max-Planck-Institut für Hirnforschung in Cologne, West Germany, transplantable neurogenic tumors of the mouse have been established, and ultrastructural and biochemical characterization of cultured, cloned cell lines is being undertaken. The consistent presence of S-100 protein, and of other proteins specific for the nervous system, in experimental tumors of neuroectodermal origin is demonstrable by fluorescent antibody and by complement fixation. Efforts are underway to use these immunochemical procedures to confirm or exclude the proposed neuroectodermal origin of both rat and mouse tumors, the nature of which is uncertain, including the subcutaneous neurofibroma/histocytoma of the extremities in both species, primary non-myogenic tumors of the heart in rats, and primary neurinoma/histocytic sarcoma of the uterus and other sites in mice.

Transplacental carcinogenic studies in the Old World monkey, Erythrocebus patas, have shown that pregnant female tolerate, without interruption of pregnancy, at least 6 successive biweekly intravenous injections of 1-ethyl-nitrosourea, each of 0.1 mg ENU/kg body weight, for a total weight-adjusted dose which is more than that necessary to induce a 100 per cent incidence of nerve tumors in rats. Efforts to increase this dosage continue. No tumors of any kind have been observed in monkeys exposed to this regimen in utero and observed up to 24 months after birth, or necropsied after this length of time following death from infectious disease. On the basis of this data it seems unlikely that ENU will induce a high incidence of the pediatric types of human neoplasms appearing early in postnatal life. To exclude the possibility that a much different proportion of the dose of ENU given the mother reaches the fetal monkey compared with the fetal rat, current efforts are directed toward study of pharmacodynamics of maternal/fetal distribution, tissue binding, and release of ENU-ethyl-^{14}C, and investigation of the relative doses required for acute (teratogenic) damage to the CNS in the rat and monkey during organogenesis.

Some transplacentally induced lung tumors in mice of genetically resistant strains are highly immunogenic in the strain of origin, grow preferentially in F_1 hybrids between the parental strain of origin and genetically susceptible strain A, are cross-reactive with normal lung tissue of strains of mice genetically susceptible to these neoplasms, and appear to be cross-reactive with normal embryonic tissue of genetically resistant strains of mice. This phenomenon provides an extremely useful system for evaluating the role of the immune response in transplacental carcinogenesis. Carcinogenic damage is inflicted before functional maturation of the immune system occurs, so that tolerance to tumor antigens may develop if these appear soon after exposure

to carcinogen: The carcinogens themselves are immune suppressive in adults, and may retard immune maturation or impair its function in the fetus. The antigenic properties of transplacentally induced epithelial tumors of liver, lung, and kidney are being actively investigated in this context.

Combination of such soluble polycations as poly-D or L-lysine with poly I: poly C yields complexes which have been shown to be extremely effective interferon inducers in primate species, since they efficiently protect the synthetic RNA polymer from degradation by serum nucleases. Collaborative studies with Drs. H. Levy and S. Baron, NIAID, are continuing on the feasibility of such polymer complexes for therapy of life-threatening viral infections in man. Since polycations are a component of this complex, further study of their carcinogenic activity in rodents is being continued. Although polylysines are less active than DEAE-dextran as sarcomagenic agents in mice, their activity is still appreciable. Furthermore, studies on the ability of the most active interferogenic polymer complexes to abort potentially lethal viral infections in primates (notably simian hemorrhagic fever in Rhesus monkeys) have so far been disappointing, since the infections could only be limited when therapy was begun before viral replication became established, and without inducing an immune response to the virus.

Tumor Pathology Section - "(1) Establishes and maintains a National Tumor Pathology Reference Center with emphasis on experimental tumors and the relationship to the human counterpart; (2) provides diagnostic pathology criteria, standardization, and monitoring for the Carcinogenesis program; (3) maintains a registry of experimental cancers, and also provides guidance and training in tumor pathology for the program and consultation services to other national and international agencies on animal tumor pathology." Pathologic evaluation of tumor development is a critical end-point that requires standardized and thorough procedures, particularly in the interpretation of carcinogenesis bioassays. The Tumor Pathology Section has developed and implemented a bioassay pathology protocol which was adopted by the Bioassay had major impact upon manpower and resource needs at both professional and technical levels in the collaborative program. This protocol is designed to assure the quality standards and the level of pathology effort necessary for the objectives of a national program on carcinogenesis bioassay.

As a result of the recent association of human liver angiosarcoma and occupational exposure to vinyl chloride, the Section has taken an active role in the acquisition and comparative evaluation of human and animal liver angiosarcomas. This tumor, although very rare in humans, is not uncommon in dogs. It has also been produced in rodents by chronic exposure to vinyl chloride and other carcinogens.

The Section organized a study on rat liver tumors and a workshop (held in Silver Spring, MD, on December 11-13, 1974) in order to develop a classification and nomenclature for several liver cell lesions which have caused controversy throughout the scientific community. The outcome was a clear consensus for a classification: the most significant result was a recommendation that the term "neoplastic" nodule replace the old term "hyperplastic" nodule. This decision was based upon a review of the experimental and biologic

knowledge available on the nature of these lesions. It was concluded that they represent a stage in the development of liver cancer, and at the very least, indicate an increased probability for the development of hepatocellular carcinoma.

In collaboration with the Center for Disease Control in Atlanta and the Johns Hopkins University, the Section has completed extensive animal pathology studies on the carcinogenic effects in rats of a polychlorinated biphenyl.

Selected pathology studies have been conducted on experimental tumors which were referred by scientists throughout the program. Lesions of unusual interest were presented and discussed at weekly slide conferences conducted by the Section which are attended y several pathologists from NCI, from other Institutes within NIH, and from the surrounding scientific community.

The objectives of the Registry of Experimental Cancers are (1) the storage and retrieval of pathological material and data on cancers and other lesions of laboratory animals (primarily rodents), and (2) the use of such information for research and educational purposes.

The Registry possesses a large collection of protocols, pathologic material, including histologic slides, paraffin blocks, and gross specimens of spontaneous and induced cancers and other lesions and has a total of 408 (164 acquired since the 1974 report) single or group accessions from investigators outside of NCI. Approximately 21,500 (1500 since the 1974 report) records have been prepared for coding and coded. The printouts are used by members of the Registry and by investigators who visit the Registry.

Nine investigators (five from abroad) have spent varying periods of time in the Registry studying some of the available material, and 77 investigators have come to the Registry for consultation, on single or multiple visits.

Ultrastructure Unit - This Unit conducts research in chemical and physical carcinogenesis through the use of electron microscopic techniques, advises and assists staff of the Carcinogenesis Program on proper utilization of such techniques for solution of experimental problems, and instructs technical and professional staff in the quantitative aspects of autoradiography. This Unit serves as a resource to collaborate with other Sections and Units and with other Branches. Research based on the output of this Unit is reported under the Section and Units for which the services are provided. Examples of intramural collaboration include the following projects: 1) assessing DNA repair synthesis (Experimental Pathology Branch; Lung Cancer Branch); 2) determining the binding of carcinogens to substructures of chromatin (In Vitro Pathogenesis Section, Experimental Pathology Branch); 3) studying the development of the rat prostate gland (Experimental Pathology Branch); 4) measuring the rate of cell proliferation in respiratory epithelium following exposure to carcinogens (Lung Cancer Branch); 5) studying localization of ^{3}H-carcinogens in respiratory epithelial cells by quantitative autoradiography (Lung Cancer Branch); and 6) studying localization of ^{3}H-vitamin A compounds and morphological changes caused by those compounds in cultured epidermal cells (In Vitro Pathogenesis Section, Experimental Pathology Branch; Lung Cancer Branch).

Project No. Z01-CP-04506-03-EXP
1. Experimental Pathology Branch
   Carcinogenesis, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through January 10, 1975

Project Title: Further Developmental Models of Prostatic Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Winston D. Edwards, Ph.D.

Other Investigators: Martin L. Wenk, Ph.D. and Elizabeth W. Kingsbury, Ph.D.

Cooperating Units: Litton Bionetics, Inc.
Microbiological Associates, Inc.

Man Year:

Total	: 3.5
Professional	: 3.0
Other	: 0.5

Project Description

Objectives:

These experiments are aimed at providing information about the morphology, mitotic activity, metabolic activity (DNA synthesis), and endocrinology of the prostate as they affect carcinogenesis in that organ. These are designed to aid in the development of an experimental model of prostatic carcinoma.

The principal experimental effort has been directed to organ culture of the prostate and to examination of the direct effects of carcinogens on cell populations which are of immediate relevance for development of an animal model of human prostatic adenocarcinoma.

Methods Employed:

Techniques for the transplantation of prostate pieces to the cleared 4th mammary fat pad of the Balb/c mouse (see major findings) have been developed. These prostate pieces (nodules) have been treated with the carcinogens 7,12-dimethylbenz[a]anthracene, 1-ethyl-1-nitrosourea, and 3-methylcholanthrene at the fat pad transplantation site.

Prostate organs have been maintained in culture. Seven day old prostate explants were transplanted from organ culture to the cleared 4th mammary pad. Investigation of the effects of the different carcinogens on these prostate explants is in progress.

These methods employed in early studies to provide information about the morphology and differentiation between the prostatic lobes were used in the organ culture system. These methods are mitotic index (colchicine), autoradiography, DNA turnover studies using tritiated precursors of DNA and standard DNA extraction, histology, high resolution microscopy, and electron microscopy.

A long term study of the effects of mating, hormones (prolactin and testosterone), and carcinogen (1-ethyl-1-nitrosourea, ENU) on the carcinogenesis of the rat prostate was undertaken with the cooperation of Microbiological Associates, Inc.

Final histological specimen from the experiments in which 7,12-dimethylbenz[a] anthracene and 3-methylcholanthrene have been injected directly into the in situ prostate of the F_{344} rat are now being evaluated.

Major Findings:

Morphological and histological differentiation between the prostatic lobes has been established. Knowledge of the basic (fine) structure of the prostate has been gained by examination at high resolution light microscopy and electron microscopy in pre-natal, neonatal, pre-pubertal, and adult stages. The mitotic activity of epithelial cell populations in prostatic lobes of rats (F_{344}) and mice (Balb/c) of various ages has been studied and autoradiographs of prostatic tissue labeled with tritiated thymidine to demonstrate DNA synthesis in epithelial cell populations of the prostatic lobes of F_{344} rats of the different age groups have been made. A study of weight increase of the prostate for the various age groups has also been completed. This work was undertaken with the cooperation of Litton Bionetics, Inc.

A suitable site for growing prostate transplants has been found. Pieces of prostate tissue were implanted in 2-3 week old male and female mice (Balb/c) and rats (F_{344}). Transplants survived in all hosts which were stimulated by testosterone, residual or exogenous, for as long as 21 months at which time the experiment was terminated. Histological examination showed that the epithelial height was maintained and that exogenous testosterone stimulation resulted in increased secretory activity in the prostate transplants. There was no survival of transplants in female host deprived of testosterone stimulation.

An organ culture technique to provide viable prostate explants has been developed. F_{344} rat and Balb/c mouse prostate organs cultured in the absence of testosterone were not maintained. When cultured in the presence of testosterone the epithelium of both ventral and dorsal prostate lobes were maintained

for seven days. However, regressive changes similar to those outlined for the explants cultured in the absence of testosterone were evident by the 10th day in culture. With the addition of testosterone, insulin, spermine and spermidine to the culture medium (plasma clot), the structural differentiation could be maintained for 14 days. No attempt was made to maintain these cultures beyond 14 days.

Seven day old organ cultured prostates were implanted into the cleared 4th mammary fat pads of female and male mice (Balb/c) and rats (F_{344}). Explants implanted in the cleared 4th mammary fat pad with no exogenous testosterone to the host did not survive in females. However, female recipients given testosterone stimulation and all male recipients with or without exogenous testosterone stimulation showed remarkable survival of explants cultured in the presence of testosterone survived in the fat pad site.

Of interest also are the preliminary results involving the injection of 7,12-dimethylbenz[a]anthracene, 3-methylcholanthrene, and 1-ethyl-1-nitroso-urea directly into the prostate transplant at the fat pad site. There is indication of hyperplastic changes in the epithelium as well as squamous metaplasia. However, microscopic examination of the tissues in this experiment is still in progress.

The study of the effects of mating, prolactin, testosterone, and ENU showed the development of pre-cancerous changes from early hyperplasia to marked hyperplasia and further neoplastic changes including in situ carcinoma, squamous carcinoma, adenocarcinoma, and sarcoma.

Significance to Biomedical Research and the Program of the Institute:

Despite the fact that the prostate is one of the most common sites of cancer in the male, there are at present no experimental models for this kind of cancer. The development of a suitable animal model for studies of prostatic adenocarcinoma and attempts to demonstrate similarities to and differences from the human gland are immediate goals of this project.

Proposed Course of Project:

Since the principal investigator on this project report has completed his three year fellowship, this project will be terminated.

Project No. Z01-CP-04510-02-EXP
1. Experimental Pathology Branch
   Carcinogenesis, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Induction and Development of Cancer of the Digestive System in Animals - Environmental Factors

Previous Serial Number: Same

Principal Investigator: Richard S. Yamamoto, Sc.D.

Other Investigators: Takashi Sugimura, M.D., D.M.S., Taijiro Matashima, Ph.D., Takashi Kawachi, M.D., D.M.S., and Gert Laqueur, M.D.

Cooperating Units: Institute of Medical Science, University of Tokyo
Japanese National Cancer Center Research Institute, Tokyo
Laboratory of Experimental Pathology, NIAMMD

Man Years:

Total	: 2.0
Professional	: 1.75
Other	: 0.25

Project Description

Objectives:

To explore the role of co-carcinogenic environmental factors in the development of gastrointestinal cancer. Dietary factors are probably the main source of carcinogens in digestive system carcinogenesis, and are also considered the most likely source of cofactors. The factors chosen for study are suggested from epidemiologic studies of human gastrointestinal cancer.

Methods Employed:

Known gastrointestinal carcinogens, such as N-methyl-N'-nitro-N-nitroso-guanidine (MMNG) for gastric cancer, and azoxymethane (AOM) and sym-dimethyl-hydrazine (DMH) for colon cancer, are administered to rats and mice, in combination with dietary factors (high salt, vitamin deficiency, protease inhibitors) which may affect the response of the gastrointestinal tract to these agents. All findings are confirmed with histopathology.

Major Findings:

1. Role of Protease in carcinogenesis. Naturally occurring protease inhibitors, leupeptin and antipain, were tested against the production of colon cancer by AOM to evaluate a possible role of protease in the formation of colon tumors. Leupeptin and antipain in the diet at 0.1% decreased the incidence of tumors of the colon, however leupeptin was more active than antipain.

2. Effect of leupeptin on the development of metastasis. A model system for pulmonary metastasis of Yoshida ascites hepatoma (AH 7974) was developed. Leupeptin, fed or injected, reduced metastasis formation in the lungs. Leupeptin did not prevent the establishment of ascites. Further studies showed that leupeptin did not reduce metastasis if adminstered after AH 7974 cells.

3. B_{12} deficiency in colon carcinogenesis. Rats on vitamin B_{12} deficient diet, developed very few colon and duodenal tumors as compared to rats on vitamin B_{12}-supplemented or Wayne laboratory chow.

Significance to Biomedical Research and the Program of the Institute.

The high morbidity and mortality from gastrointestinal cancer is a major health problem in the United States. The development of animal models of gastrointestinal neoplasms, and their use for the study of etiological factors which act in combination of factors which modify the incidences and development of such tumors, is expected to lead to knowledge relevant to their prevention.

Proposed Course of Project:

Further studies will be made with dietary ingredients, antioxidants, antibiodics, resistant vs. susceptible strains and species, enzyme inhibitors, etc., to explore their effects on carcinogenesis by compounds known to affect the gastrointestinal tract. The new model system of hamsters with the newly demonstrated pancreatic carcinogen, 2,2-dihydroxypropanenitrosamine, will be added to the studies of gastrointestinal carcinogenesis.

Honors and Awards

Member, Committee on Standards for Nutritional Studies, American Institute of Nutrition.
Participant, Workshop, Colon Cancer Segment, Carcinogenesis Program.

Publications

Matsushima, T., Yamamoto, R. S., Hara, K., Sugimura, T., Takeuchi, T., and Umezawa, H.: Azoxymethane ni yoru daicho-gan hassei no leupeptin ni yoru yokusei (Inhibition of azoxymethane-induced colong cancer by leupeptin). Igaku no ayumi 88: 710-714, 1974.

Ward, J. M., Yamamoto, R. S., Benjamin, T., Brown, C. A., and Weisburger, J. H.: Experimentally induced cancer of the colon in rats and mice. J. Am. Vet. Med. Assoc. 164: 729-732, 1974.

Matsushima, T., Yamamoto, R. S., Hara, K., Sugimura, T., Takeuchi, T., Ward, J. M., Weisburger, J. H., Yamamoto, R. S., Benjamin, T., Brown, C. A., and Weisburger, E. K.: Long-term effect of benzene in C57BL/6N mice. Arch. Environ. Health 30: 22-25, 1975.

Project No. Z01-CP-04502-04-EXP
1. Experimental Pathology Branch
Carcinogenesis, DCCP
2. In Vitro Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Role of DNA Replication and Repair in Chemical Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Henry Hennings, Ph.D.

Other Investigators: Delores Michael and Elroy Patterson

Cooperating Units: None

Man Years:

Total	2.2
Professional	1.0
Other	1.2

Project Description

Objectives:

To examine the chemical carcinogen-induced repair of DNA in cultured rodent and human cells; to examine the importance of cell proliferation in skin carcinogenesis initiated by urethane, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 7,12-dimethylbenz(a)anthracene (DMBA) or β-propiolactone.

Methods Employed:

In most experiments, cell cultures of newborn mouse skin or epidermis were grown in Medium 199 in petri dishes. The cells were lysed in 1% sarkosyl, homogenized, and centrifuged in a cesium chloride-cesium sulfate gradient in a Beckman L2-65B ultracentrifuge to separate DNA from RNA and protein. In biochemical experiments in mouse skin, the animals were sacrificed, and the back skin was removed and put into cold 1% acetic acid overnight. The epidermis was then scraped off and was used for preparation of a nucleic acid hydrolysate by cold perchloric acid (PCA) precipitation of macromolecules and hot PCA hydrolysis. A similar PCA procedure was employed for studying rates of thymidine-^{3}H incorporation in epidermal cells in culture.

Major Findings:

DNA repair: DNA repair was studied by the BUdR-CsCl centrifugation technique in primary cultures of mouse skin cells treated with the carcinogen MNNG. When both BUdR and a tritiated DNA precursor are present during DNA repair synthesis, tritium incorporated into light density, unreplicated DNA indicates DNA repair. Repair was shown with all deoxyribonucleoside precursors tested after treatment for 1 hr with high, toxic doses of MNNG (25-50 μg/ml). However, at lower doses of MNNG (1-5 μg/ml), repair synthesis was detected only with GdR^{3}H. Paper chromatography of enzymatic hydrolysates of GdR-^{3}H-repaired DNA indicated that 99.5% of the tritium was incorporated into DNA guanine. Doses of 1, 5 or 25 μg/ml MNNG reduced DNA synthesis at 1-2 hr by 19%, 62% and 96% and decreased the amount of DNA per dish at 26 hr by 12%, 32% and 69% respectively, thus indicating significant toxicity with the highest dose.

The apparently specific repair seen at lower, less toxic doses of carcinogen may be more relevant to cell survival than the non-specific repair seen at very toxic doses.

After a one hour treatment of cultured mouse epidermal cells with 25 μg/ml MNNG, the level of repair shown with thymidine-^{3}H was 58% greater at 1-5 hours than at 24-28 hours. In contrast, after a dose of 5 μg/ml MNNG, the level of repair demonstrated with deoxyguanosine-^{3}H was 31% higher at 24-28 hours than at 1-5 hours. This difference in kinetics between the 2 types of repair suggests that they are distinct, separate processes.

DNA repair synthesis was examined with TdR and GdR at 2 or more doses of MNNG in mouse epidermal cells, mouse fibroblasts rat skin cultures, rat epidermal cells and WI-38 human fibroblasts. GdR-specific repair was found in all rodent cells examined, but in WI-38 cells repair was demonstrated equally well with both precursors at all MNNG doses. Thus, GdR-specific repair could not be shown in a line of human cells.

Cell Proliferation. Cell proliferation appears to be important in the initiation of skin tumor formation, but its exact role remains to be elucidated. A single treatment with the hyperplastic agent croton oil at times for 24 hours before until 12 hours after an initiating injection of urethane increased the papilloma incidence in initiation-promotion experiments 2-3-fold. Croton oil treatment 24 hours after urethane injection had no enhancing effect. With MNNG as initiator, croton oil treatment at all times tested from 6 hours before until 48 hours after initiation increased the papilloma yield 2-3-fold.

Tritiated thymidine incorporation into epidermal DNA in mice treated with croton oil 6 hours before or 1 hour after initiation with urethane or MNNG was examined. Croton oil alone increased the rate of DNA synthesis beginning at 12-15 hours, reaching a peak at 18-24 hours, and remaining above control values for 4-5 days. Urethane alone inhibited DNA synthesis by 50% for about a day, with a slight stimulation at 2-3 days. When croton oil was given 6 hours before urethane, DNA synthesis was increased above the control value by 8 hours after initiation, reached a peak at 18 hours, decreased,

increased to a second peak at around 50 hours and remained 2.5-fold above controls at 4 days. When croton oil was given 1 hour after urethane, a similar response was found, with the expected delay of a few hours. After MNNG treatment, thymidine-^{3}H incorporation was decreased to 20% of the controls at 12 hours, with a gradual increase to 1.5 times control values by 3-5 days. When croton oil was given 6 hours before or 1 hour after MNNG, a small first peak of DNA synthesis at 18-24 hours was followed by greatly increased DNA synthesis at 2-5 days after MNNG.

The croton oil-induced replication of DNA damaged by urethane or MNNG could result in permanent DNA alterations by the fixation of oncogenic mutations in the DNA of daughter cells before the damage could be repaired. The rate of DNA repair in rapidly-proliferating cells could differ from that in cells with a normal proliferation rate. However, preliminary results in epidermal cells in culture indicate that the extent of DNA repair demonstrated with GdR or TdR after low or high doses of MNNG was similar when cells with a low rate of DNA synthesis were compared to cells with 30-35 times the level of thymidine-^{3}H incorporation. Croton oil was found to inhibit DNA repair (in addition to its early inhibition of DNA replication) in epidermal cell culture only when given prior to an alkylating agent, so it appears unlikely that the enhancement of tumor formation when given after MNNG or urethane is due to inhibition of DNA repair.

An attempt is being made to find derivatives of urethane which will be active when added to cells in culture. Doses of urethane as high as 2 mg/ml medium were not toxic to epidermal cells. However, when ethoxycarbonyloxyurethane was added to cultures of epidermal cells, levels of 0.5-10 µg/ml inhibited thymidine-^{3}H incorporation by 50-90% within 2 hours and induced DNA repair demonstrated with deoxycytidine-^{3}H as precursor.

Rates of DNA synthesis have been determined at frequent intervals during the first 3 days after plating primary cultures of mouse epidermal cells. Thymidine-^{3}H incorporation was low for the first 18 hours in culture, but then increased rapidly to large peaks at 35-40 hours and around 60 hours. Peak values were 30-35 times higher than those found at 11-15 hours after plating. Similar differences in rates of DNA synthesis have been demonstrated when comparing thymidine-^{3}H incorporation in confluent cells remaining in old medium for 6 days with cells which were left in old medium for 5 days, then stimulated by the addition of fresh complete medium. These differences in rates of DNA synthesis will be utilized to compare the effects of carcinogens on DNA repair and possibly transformation of the cells.

Significance to Biomedical Research and the Program of the Institute:

If a carcinogen-induced alteration in DNA is necessary for carcinogenesis, then the repair of the critical alteration should reduce subsequent tumor formation. Replication of the DNA prior to repair may result in a permanent non-repairable change in the base sequence of the newly-synthesized DNA. Conversely, errors

in the repair process could also lead to permanent DNA alterations. Our experiments are designed to elucidate the roles of DNA replication and DNA repair in chemical carcinogenesis.

Proposed Course of Project:

The detailed kinetics of guanine-specific repair after low doses of MNNG will be compared with the kinetics of repair which can be demonstrated with any precursor after higher doses of MNNG.

Further derivatives of urethane, such as N-acetoxyurethane, will be tested for induction of DNA repair in epidermal cells in culture. The urethane derivatives will also be tested as initiators in initiation-promotion experiments.

The effect on rates of synthesis of DNA and RNA of treating newborn mice with the tumor promoter tetradecanoyl phorbol acetate (TPA) is currently being determined.

Tumor induction experiments are being started to determine the effect on tumor development of a single application of acetic acid (a hyperplastic agent which is a weak tumor promoter) given soon after urethane or MNNG. In addition, it will be determined whether the enhancement of urethane or MNNG initiation by an application of croton oil soon after initiation also occurs with other initiators such as DMBA or β-propiolactone. The specificity of several treatments which have been reported to inhibit promotion will be determined by testing their effect on initiation also.

A system for studying DNA repair in confluent epidermal cells similar to that demonstrated in lymphocytes and WI-38 cells is being set up. This would allow the rapid estimation of repair with several different precursors after treatment with a carcinogen.

Publications

Hennings, H., Micahel, D., Eaton, S., and Morgan, D. L.: Purine-specific repair of β-Propiolactone-induced DNA damage in mouse skin cells. J. Invest. Dermatol. 62: 480-484, 1974.

Project No. Z01-CP-04504-03-EXP

1. Experimental Pathology Branch
   Carcinogenesis, DCCP
2. In Vitro Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Model Systems for the Study of Chemical Carcinogenesis at the Cellular Level

Previous Serial Number: Same

Principal Investigator: Stuart H. Yuspa, M.D.

Other Investigators: Kjell Elgjo, M.D.
Ulrike Lichti, Ph.D.
David L. Morgan, B.S.

Cooperating Units: Lung Cancer Branch

Man Years:

Total	3.0
Professional	3.0
Other	0

Project Description

Objectives:

To develop model systems which can be utilized to study cellular events which occur during early stages of chemical carcinogenesis. Systems studied are directed to give both general information regarding malignant transformation within a mammalian cell and specific requirements of specialized cells which might shed light on events necessary for tumor development in a particular organ.

Methods Employed:

Systems have been developed for studying epithelial cell transformation in vitro. A model system of mouse epidermal cell culture which parallels the in vivo model of mouse skin carcinogenesis has received most attention. Newborn mouse skin is floated overnight on a cold trypsin solution which separates dermis from epidermis. Epidermal cells can then be dissociated and grown as a monolayer. Hair follicles and dermal fibroblasts can also be isolated and grown in culture. Epidermal cells can be grafted back to syngeneic hosts as a skin graft on graft beds prepared several weeks earlier

and protected by silicone domes and wire screens. Epidermal cells can be treated by chemical and viral carcinogens in vitro and continued in vitro for long-term studies or grafted back to hosts for in vivo passage. In addition promoting agents can be applied to epidermal cells in vitro. Treated cultured cells are evaluated by several criteria: morphology by both the light and electron microscope, growth in agar ability to induce fibrinolysis and injection back into mice. Since newborn epidermal cells are utilized, pregnant mice can be injected with carcinogens and the epidermis of the offspring cultivated in vitro. This allows a comparison of normal and initiated epithelial cells in vitro as well as an approach to the study of promotion in vitro. Epidermal cell kinetics are also being studied using radioactive labeling with DNA precursors and isolation of DNA or autoradioagraphy.

Epidermal cell differentiation is being studied in this system in order to relate this phenomenon to carcinogenesis. Vitamin A treatment of these cells caused a marked alteration in differentiation. This is observed by light and electron microscopy, DNA turnover studies, and altered glycoprotein synthesis. Studies of the biochemical interaction between chemical carcinogens and Vitamin A altered cells are performed to evaluate alterations in the response to carcinogens in various states of differentiation. Ornithine decarboxylase, an enzyme involved in early cellular response to stimuli affecting proliferation or differentiation is being studied in this epithelial system. The interaction of chemicals with cellular macromolecules is being studied in isolated chromosomes or nuclei. Chromosomes or nuclei are isolated from Chinese hamster cells and processed into sub units by various procedures. Binding of carcinogens to these subunits are studied using ultracentrifugation, spectrophotometric, and radiotracer techniques.

Major Findings:

Mouse epidermal cells, free of fibroblasts, can be grown in vitro and will keratinize as they do in vivo. These cells can be grafted back to syngeneic F_1 hybrid hosts and will form skin characteristic of the donor strain. Cell kinetic studies of epidermal cells in vitro suggest a pattern of partially synchronized DNA synthesis. Approximately 60% of the cells are proliferating. Synchronous peaks of DNA synthesis involving up to 50% of the cycling cells occur at 20, 40, and 60 hours after plating. Total cell number decreases gradually as cells keratinize and slough during the first 2 weeks in culture Ultrastructurally these cells contain tonofilaments, desmosones and granules suggestive of keratohyalin. Cells from early primary cultures will bind polycyclic hydrocarbon carcinogens to cellular macromolecules 2-4 times more than 10 day old cells. There is a similar decrease in aryl hydrocarbon hydroxylase (AHH) activity with age in vitro. Both binding and AHH activity can be preserved by culturing cells in the presence of 1.25% DMSO. The promoting agent for skin carcinogenesis, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), stimulates epidermal DNA synthesis by 5-10 fold within 72 hours of application in vitro. This response occurs over a wide dosage range and with an exposure time of less than 1 hour. Dermal fibroblasts do not respond to TPA treatment in vitro.

Ornithine decarboxylase (ODC), a key enzyme in the formation of polyamines, is stimulated 10-20 fold within 10 hours of TPA application to epidermal cells and gradually returns to baseline levels over the next 24 hours.

If pregnant mice are treated intraperitoneally with urethane and their offspring used as a source of epidermal cells, the pattern of DNA synthesis is altered. There is a stimulation of replication and the lifespan of epidermal cell cultures is increased. Combined treatments of urethane in vivo and TPA in vitro has yielded cell lines apparently transformed morphologically and biologically. One such line produces undifferentiated carcinomas when injected into newborn mice and high levels of plasminogen activator when assayed fibrinolysis.

Retinyl acetate (RA) added to the tissue culture medium in DMSO causes marked alteration in epidermal cell differentiation. Keratinization is inhibited. There is a marked increase in glycoprotein synthesis with production of a new species of glycoprotein as compared to controls. Although DNA synthesis is not increased, total cell number remains unchanged or increases in the presence of RA suggesting a prolonged lifespan. Repair of DNA damage caused by ultraviolet light or bromomethylbenzanthracene is not altered in RA treated cells. Retinyl acetate promotes binding of DMBA to epidermal proteins but diminishes binding to DNA when compared to DMSO controls. AHH activity is decreased by RA treated cell when compared to DMSO controls. RA remains bound to epidermal cells for up to 7 days after a single 17 hour exposure.

Chinese hamster chromosomes can be isolated in large numbers from cells in mitosis, harvested after a colcemid block. Polycyclic hydrocarbon carcinogens will bind to the macromolecules of these chromosomes. Chromosomes can be fractionated by size, or alternatively, chromosomes can be fractionated into subunits by salt extraction. This technique yields a structural chromosomal unit and a functional chromosomal unit, the former being heterochromatic and the latter euchromatic. Studies to investigate whether preferential binding of carcinogens occurs in a chromosomal subunit are preliminary but suggest that benzopyrene preferentially binds to heterchromatin while DMBA binds equally well to both.

Significance to Biomedical Research and the Program of the Institute:

It is essential to understand early changes at the cellular level which occur during initiation of malignant change in order to prevent this transformation from occurring or to revert cells from an initiated state back to normal. The use of the epidermal cell culture system developed in this laboratory has certain advantages in approaching this problem. It utilizes epithelial cells which have shown to be normal in terms of differentiation and ability to perform properly when placed back into its environment in the whole animal. Since the vast majority of human tumors are of epithelial origin, this epithelial system represents an important model for chemical carcinogenesis.

The development of an in vitro skin carcinogenesis model allows for the continued study at a cellular level of extensive in vivo data which could not be carried further due to limitations of the whole animal. The separation of skin components allows for the first time a model to determine which cell types (i.e. hair follicle, basal cell, fibroblasts) are essential for the transformation to occur. With transplantation at an observable animal site performed at an early state in the in vitro life of the cells, the long-term ambiguities of cell culture can be avoided. The remarkable response of epidermal cells to TPA adds a new dimension to cell culture studies of carcinogenesis - the study of promotion. Two stage in vitro carcinogenesis can now be investigated. In addition this system can be useful for studying biochemical steps involved in initiating cellular proliferation in general.

The use of retinyl acetate in this culture system allows a systematic approach to the study of differentiation and vitamin A effects. There is a growing but ambiguous literature on both vitamin A effects on carcinogenesis and the relationship between differentiation and carcinogenesis. This should be amenable to study using this epidermal cell culture system. Evidence from other systems suggest that vitamin A may be able to reverse the process of malignant transformation in initiated cells. If this process could be understood or studied more extensively it might offer an opportunity to alter the prognosis for some categories of human individuals at high risk for carcinoma.

The combined use of transplacental treatment of epidermal cells with in vitro observation and manipulation allows several potentially important new uses for this system. The process of promotion can be studied in vitro in cells initiated prior to culture. Preliminary evidence suggests that the addition of a second carcinogen in vitro causes a rapid transformation to occur. This is potentially useful then as an assay system for both promoting agents and initiating agents.

There is increasing evidence that chemical carcinogens alter genetic material. Chromosomes and sub-chromosomal units offer a way of studying intact genetic information. The effects of chemical agents on those structures should be of great interest.

Proposed Course of Project:

Epidermal cells will be used to study carcinogenesis in vitro or by combined in vitro and in vivo techniques. The role of state of differentiation, proliferative capacity, and cell cycle will be studied. Both normal and preinitiated cells will be used. Phorbol ester will be added to normal and initiated cells to study promotion in vitro and cellular proliferation. The role of polyamine biosynthesis in relation to cellular proliferation and differentiation will be studied.

The role of vitamin A in carcinogenesis will be studied in this system. An attempt will be made to separate effects on initiation from effects on promotion. Parallel in vivo studies will be performed.

Binding of carcinogens to informational subunits of the cell will be studied further. When studies on chromosomal subunits are completed, groups of chromosomes will be isolated by size and binding will be studied. In addition the interaction of carcinogen with functional subclasses of DNA will be investigated.

Publications

Yuspa, S. H. and Harris, C. C.: Altered differentiation of mouse epidermal cells treated with retinyl acetate in vitro. Exp. Cell Res. 86: 95-105, 1974.

DeLuca, L. and Yuspa, S. H.: Altered glycoprotein synthesis in mouse epidermal cells treated with retinyl acetate in vitro. Exp. Cell Res. 86: 106-110, 1974.

Project No. Z01-CP-04508-03-EXP
1. Experimental Pathology Branch
Carcinogenesis, DCCP
2. In Vitro Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Effects of Tumor Promoters and Non-promoting Analogs on DNA Repair Synthesis

Previous Serial Number: Same

Principal Investigator: Miriam C. Poirier, M.S.

Cooperating Units: None

Man Years:

Total	:	1.0
Professional	:	1.0
Other	:	0.0

Project Description

Objectives:

Reports in the literature have suggested that some co-carcinogens and tumor promoters inhibit DNA repair synthesis. Because of the potential importance of these observations, the effects of some well-known tumor promoters, and non-promoting chemical analogs, on DNA repair synthesis were investigated.

Additional studies investigating the effects of the same promoters and analogs on DNA replicative synthesis, RNA and protein synthesis and cell morphology, were designed to study the specificity of the effect on DNA repair synthesis.

Methods Employed:

Confluent WI-38 cells (human diploid fibroblasts), given 10^{-2}M Hydroxyurea to suppress any DNA replicative synthesis, were damaged with 10^{-5}M NA-AAF and allowed to repair in the presence of ^{3}H-TdR for 4 hours. Repaired DNAs were analyzed by CsCl gradient density centrifugation. Tumor promoters or analogs were administered before the NA-AAF damage. ^{3}H-TdR-labeled, replicated DNAs were also analyzed on CsCl gradients.

RNA and protein were separated on mixed CsCl-Cs_2SO_4 gradients. After dialysis RNAs were determined by A_{260} and proteins by Lowry. For morphology studies WI-38 cells were grown on cover slips, treated with promoters or analogs, fixed and stained with Giemsa.

Major Findings:

Over a range of doses, three promoters [croton oil, 12-O-tetradecanoyl-phorbol-13-acetate (TPA) and anthralin] were found to inhibit DNA repair synthesis while their non-promoting analogs [phorbol and 1,8 dihydroxyanthraquinone] had little effect. Another tumor promoter, phenol, inhibited DNA repair synthesis only at very high concentrations while an analog, 4-nitrophenol, produced inhibition of DNA repair synthesis at molar concentrations at which phenol had no effect.

To investigate the specificity of this phenomenon, the effects of these agents on DNA replicative synthesis, RNA synthesis, protein synthesis, and cell morphology were evaluated. At equimolar concentrations, tumor promoters were found to inhibit DNA replicative synthesis as effectively as repair synthesis. RNA and protein synthesis were similarly inhibited over the same range of concentrations. Extensive morphologic changes, interpreted as evidence of toxicity, were seen at concentrations of promoters that inhibited the macromolecular syntheses studied. The non-promoting analogs, with the exception of nitrophenol, had little effect on these processes and showed only slight morphological damage.

In conclusion, it was shown that DNA repair synthesis is inhibited in WI-38 cells at concentrations very similar to those observed in other laboratories with other cell lines; at these same concentrations DNA replication, RNA synthesis, and protein synthesis are also inhibited, and cell morphology is altered.

Thus tumor promoting agents appeared to inhibit a number of macromolecular synthetic events, including DNA repair synthesis. It is suggested that the effect of tumor promoters on DNA repair synthesis is part of a general response to cellular injury rather than a selective response involving a single metabolic pathway.

Significance of Biomedical Research and the Program of the Institute:

The process of DNA repair synthesis, after damage with chemical carcinogen, would seem to be a major mechanism by which the cell attempts to maintain its genomal integrity, and thus minimize carcinogenesis. The idea that classical promoting agents might exert their effect by an inhibition of DNA repair synthesis is particularly attractive. A few groups have reported inhibition of DNA repair synthesis by promoting agents, but none have investigated the specificity of the effect in relation to other metabolic processes.

The present study leads to the conclusion that mechanism of tumor promotion probably does not involve specific inhibition of DNA repair synthesis.

Proposed Course of Project:

The project reported here is essentially terminated, and a manuscript has been accepted for publication. Similar studies have been planned using primary mouse epidermis cultures, and concentrations of hydroxyurea adequate to suppress DNA replicative synthesis in these cells have been determined. However attempts to find DNA repair synthesis using N-acetoxy-acetylaminofluorene, 7-bromomethylbenz[a]anthracene, and UV light in this system have not been successful.

Publications

Poirier, Miriam C. and Lieberman, Michael W.: Non-specific inhibition of DNA repair synthesis by tumor promoters in human diploid fibroblasts damaged with N-Acetoxy-2-Acetylaminofluorene. Cancer Res. (In Press).

Lieberman, M.W. and Poirier, M. C.: Base pairing and template specificity during deoxyribonucleic acid repair synthesis in human and mouse cells. Biochemistry 13: 5384-5388, 1974.

Lieberman, M. W.: Approaches to analysis of fidelity of DNA repair in human diploid cells. Int. Rev. Cytol. suppl. 1974 (In Press).

Project No. Z01-CP-04503-03-EXP
1. Experimental Pathology Branch
Carcinogenesis, DCCP
2. Perinatal Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunological Factors Responsible for the Differential Sensitivity Between Fetal and Adult Mice, and Between Different Inbred Strains, to the Induction of Pulmonary and other Epithelial Tumors

Previous Serial Number: Same

Principal Investigator: Jerry M. Rice, Ph.D.

Other Investigators: W. John Martin, M.B., Ph.D.

Cooperating Units: Immunology Section, VLLB, VO, NCI

Man Years:

Total	:	0.6
Professional	:	0.1
Other	:	0.5

Project Description

Objectives:

To determine the manner and the extent to which immunological factors are responsible for the genetically determined differences in sensitivity to the induction and growth of epithelial tumors among different inbred strains of mice, and between the fetus and adult within each strain.

Methods Employed:

Tumors are induced transplacentally by treatment of timed-pregnant mice with 1-ethyl-1-nitrosourea (ENU), which is synthesized in this laboratory. Tumors are transplanted to secondary hosts using trocar implants or counted cell suspensions, and to serial cell culture using standard media and procedures. Microcytotoxicity tests are carried out using lymph node and spleen cells from tumor bearing animals. In vivo studies of immunological cross-reaction are carried out by pre-immunizing mice with normal or tumor tissue, subsequently suppressing the primary (but not the secondary) immune response by 400 R whole-body X-irradiation, and challenging with the tumor to be tested. Masked cell surface antigens are exposed by digestion of viable cells with neuraminidase or other enzymes.

Major Findings:

It has previously been reported that some pulmonary tumors induced transplacentally in strains of mice genetically resistant to these tumors grow poorly or not at all on transplantation to syngeneic recipients, but grow readily in F_1 hybrids between the strain of origin and another strain genetically more susceptible to the induction of the same type of tumor. Two such tumors (designated 38 and 85) from strain C3Hf, which is moderately resistant to pulmonary tumor induction, behave in this fashion and have been shown previously to be cross-reactive with each other and with normal lung tissue from genetically sensitive strain A, but not with histologically unrelated tumors or with lung tissue from genetically resistant strains. Extension of these studies to approximately 40 new transplacentally induced lung tumors in strains C3Hf, DBA/2, and C57Bl/6 (all resistant to lung tumor induction and growth) is now in progress. Studies are being undertaken to determine whether the common antigen in strain A lung tissue and in lung tumors 38 and 85 can be unmasked in most or all lung tumors by treatment with neuraminidase and other enzymes. It has been established that expression of the cross-reactive antigen is a stable trait, persisting in transplanted tumors for at least 20 generations and in cultured cells derived from them for at least 2 years. Preliminary studies indicate that immunization of C3Hf mice against tumor 85 can be effected by normal fetal, but not adult tissues of lung tumor resistant strain C57Bl/6. This suggests that the antigen behaves like many other types of carcino-embryonic antigens, in that it is normally expressed at some time during development, but appears in adult tissues only under certain abnormal conditions, especially neoplasia.

Transplacentally induced hepatocellular tumors, which occur readily in strain C3Hf and less frequently in other strains, have been transplanted from strains DBA/2, C57Bl/6, and A to recipients of the parental strain and its F_1 hybrid with strain C3Hf, to ascertain whether an inverse hybrid effect can be observed with liver tumors. One renal carcinoma from strain C57Bl/6 is similarly under investigation in comparison with inbred strain Balb/Cf/Cd, which is genetically susceptible to renal carcinoma.

Significance to Biomedical Research and the Program of the Institute:

This experimental system represents the first experimental demonstration of a role of immune factors in genetically determined sensitivity and resistance to a particular type of tumor. The existence of cross-reactive antigens which confer transplantation resistance on tumors and normal lung tissue of susceptible individuals invites the question of whether a similar mechanism may explain tolerance (susceptibility) to other types of tumors in animals or in human beings. This in turn has significance for efforts to develop immunotherapeutic measures against neoplastic disease, since the problem of avoiding autoimmunization must be faced. This experimental model provides

the means for exploring the significance of that problem, as well as for determining whether non-specific stimulation of immune responsiveness will suffice to initiate tumor rejection in individuals who are intrinsically tolerant to tumor antigens.

Proposed Course of Project:

The neuraminidase studies with lung tumors newly isolated from resistant strains should be emphasized in order to establish how generally the cross-reactive antigen is distributed. Extension of these studies to other tumors of other organ systems, notably liver and kidney, is being attempted; these have been chosen to reflect a pattern of susceptibility and resistance among mouse strains which differs from the lung tumor pattern. The studies of cross-reactivity should be extended to determine the tissue localization and time of appearance of cross-reactive antigens during ontogeny. In collaboration with laboratories interested in immunotherapy, efforts will be made to exploit the cross-reactive immunity of tumor 85, in combination with non-specific immune stimulants, in an experimental immunotherapy system, first with "artificial" lung tumors induced by intravenous injection of tumor 85 cells, later with autochthonous chemically induced tumors. Careful attention will be paid to the possibility of autoimmune pulmonitis and other manifestations of autoimmune disease in such immunized mice.

Project No. ZO1'-CP-04687-05-EXP

1. Experimental Pathology Branch
   Carcinogenesis, DCCP
2. Perinatal Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Transplacental and Neonatal Carcinogenesis, including Experimental Models of Human Childhood Neoplasms

Previous Serial Number: Same

Principal Investigator: Jerry M. Rice, Ph.D.

Other Investigators: Sewa R. Joshi, B.V.Sc., Ph.D., Larry Keefer, Ph.D., Peter Roller, Ph.D., William T. London, D.V.M., Ray Shenefelt, M.D., Martin Wenk, Ph.D., Wolfgang Wechsler, M.D., and S. D. Vesselinovitch, D.V.M., Sc.D.

Cooperating Units: NINDS; Carcinogen Metabolism and Toxicology Branch; National Center for Toxicological Research, Jefferson, AR; Microbiological Associates, Inc., Bethesda, MD; Max-Planck-Institut für Hirnforschung, Cologne, Germany; Meloy Laboratories, Rockville, MD; University of Chicago, IL.

Man Years:

Total	5.0
Professional	3.5
Other	1.5

Project Description

Objectives:

To develop experimental animal models for human cancers of childhood, and to study the greater sensitivity of fetal and neonatal animal tissues to chemical carcinogens, as well as the roles of factors other than the carcinogens themselves in enhancing or suppressing chemical carcinogenesis in experimental animals during intrauterine life and infancy.

Methods Employed:

Carcinogenic organic compounds are synthesized where necessary; organic polymers are obtained commercially and characterized spectroscopically,

and purified where necessary. Animals of various species and strains, including non-human primates, are exposed to these compounds at different times during pre- and postnatal life, and the carcinogenic response in various organs is evaluated histologically. Short and long-term cell and organ culture techniques are used where necessary to explore the role of host defense mechanisms in resistance to carcinogenesis, and to study cocarcinogenic effects of microbial and viral infections in chemical carcinogenesis. Surgical procedures, including skin grafting, adult thymectomy, etc., are used in evaluating antitumor defense mechanisms of the intact animal, and organic and biological chemical techniques are employed to determine the effects of modifications of chemical carcinogen structure and of metabolic factors on the response of host tissues to carcinogens. Organ culture techniques are being undertaken to determine the ability of apparently mesenchymal rat kidney tumors to respond to embryological inducers of renal differentiation.

Major Findings:

In contrast to its predominantly neurotropic effects in rats, the carcinogen 1-ethyl-1-nitrosourea (ENU) induces chiefly epithelial tumors of the lung, liver, and other viscera when given transplacentally to mice. Approximately 100 neurogenic tumors have, however, been observed in ENU-treated mice (an incidence below 5% of animals at risk). These have been classified, and in some cases established as transplantable tumor and continuous cultured cell lines, in collaboration with Dr. Wolfgang Wechsler of the Max-Planck-Institut für Hirnforschung, Cologne, Germany. S-100 and other proteins specific for the nervous system are uniformly present in these tumors and in cultured cell lines prepared from them, and persist through at least one year of monthly subculturing in cell lines and through at least 10 generations in transplanted tumors.

However, transplacental 7,12-dimethylbenz[a]anthracene in the Sprague-Dawley rat not only induces a high proportion of neurogenic tumors, but inflicts a selective, permanent depletion of small neurons of the internal granular layer of the cerebellum, which invites the prediction that potential medulloblastoma cells should exist among the survivors. In vitro procedures to identify such cells are in progress. This work is proceeding in collaboration with Dr. R. Shenefelt, NCTR.

Renal tumors induced by ENU in mice, transplacentally or at any later stage of life, are exclusively of epithelial origin and adult morphology, while the majority of renal tumors induced in rats either transplacentally or after birth and with either aromatic hydrocarbons or nitrosamine derivatives are primary renal sarcomas. There are some true nephroblastomas (mixed poorly differentiated epithelial and mesenchymal tumors) among the rat tumors, however, and the possibility exists that at least some of the "sarcomas" represent an extreme variant of the nephroblastoma in which the epithelial component is vanishingly small. In vitro studies on such tumors

using the Grobstein technique of co-cultivation with embryonic mouse neural tube have not yet succeeded in inducing epithelial differentiation, as can be done in this way with human embryonal nephromas of similar morphology. Experiments are continuing with improved media recently described for other epithelial/mesenchymal systems where differentiation must occur.

The extremely weak transplacental carcinogenic activity of the dialkylnitrosamines, such as dimethylnitrosamine (DMN), in marked contrast to the powerfully active alkylnitrosoureas, has been ascribed to the failure of fetal tissues to provide the enzyme-mediated metabolic conversion of DMN and related compounds to chemically reactive carcinogenic metabolites. Synthesis of stable derivatives of α-hydroxy dialkylnitrosamines has been achieved by Drs. L. Keefer and P. Roller of the Toxicology and Metabolism Branch, and in collaboration with them the comparative toxicity and carcinogenicity of DMN, methyl(acetoxymethyl)nitrosamine (DMN-OAc) and methyl(methoxymethyl)-nitrosamine (DMN-OMe) are being established in fetal, newborn, and adult Sprague-Dawley rats. DMN-OAc has proved to be a specific and highly potent carcinogen for the small intestine and colon when given to weanlings as a single intraperitoneal injection, yielding approximately a 40% incidence of single or multiple intestinal carcinomas within 13 months. Its patterns of tissue distribution, bile/urine excretion, and tissue binding are currently under investigation using DMN-OAc-methyl-^{14}C synthesized by Dr. Roller. By contrast, DMN-OMe induces renal tumors exclusively, reflecting the requirement for oxidative enzyme-mediated activation characteristic of DMN.

Transplacental carcinogenesis studies in the Old World monkey, Erythrocebus patas, have shown that pregnant animals and their fetuses tolerate up to 6 sequential intravenous injections of ENU, at a single-dose level of 0.1 mmole ENU/kg maternal body weight. This total dose is at least 10 times that necessary to induce a 100 per cent incidence of nerve tumors in rats. However, no tumors of any kind have yet been observed in monkeys exposed to ENU in utero and observed up to 24 months after birth. Synthesis of ENU-ethyl-^{14}C is now in progress, to be used in defining the maternal/fetal distribution, tissue binding, and elimination of ENU from the patas in comparison with the highly susceptible Sprague-Dawley rat. Comparative teratogenic effects of ENU in these species are being evaluated. The patas colony is maintained at Meloy Laboratories, and the project is collaborative with Dr. W. T. London, NINDS.

The water-soluble polycation diethylaminoethyl (DEAE)-dextran has been found to be oncogenic in the rat and possibly in the Syrian hamster, after latency periods much longer than in the more susceptible mouse. Drs. H. Levy and S. Baron, NIAID, with whom collaborative studies on interferon inducers are performed, have shown that our complex of poly (I):poly (C) with the polycation poly-D-lysine is highly effective as an interferon inducer in primates, because of the protection afforded the polynucleotide against serum nucleases. In view of the potential use of this substance as an antiviral drug in man, the nature and limitations of the oncogenic activity of soluble polycations

are continuing under study. Leukemogenic viruses isolated so far from cultured cells of sarcomas induced by subcutaneous injection of such polymers consist solely of agents that are NB-tropic at the Fv-1 locus and belong to the FMR subgroup. No focus-forming or sarcomagenic agent has been isolated. Studies are continuing on the hypothesis that the oncogenic activity of soluble polycations nonetheless involves viral-chemical interaction. Studies are also continuing on the efficacy of the most potent interferon-inducing polymer complexes against lethal viral infections in primates. Simian hemorrhagic fever can be aborted in rhesus monkeys only if replication of inoculated virus has not begun; no antibody titer develops in such animals. Other lethal primate viral infections are under investigation.

Significance to Biomedical Research and the Program of the Institute:

Research for animal models of human childhood neoplasms should provide an insight into the types of causative agents and modes of exposures responsible for childhood cancer. It is to be expected that natural selection would tend to eliminate genotypes in the human population which predispose individuals to the development of fatal neoplasms before attaining reproductive age, yet the incidence of tumors in childhood is high. Epidemiological studies have pointed to the occurrence of childhood neoplasms in association with certain types of congenital malformation which are non-inherited, and suggest that environmental agents, alone or in combination, may play a role in the induction of such neoplasms. The ability to induce medulloblastomas chemically in mice allows studies on the nature and inducibility of these tumors to proceed. The fact that such tumors are not induced in the closely related rat by identical treatment may provide an experimental approach to identifying factors which are important for their development.

Most tumors induced transplacentally in rodents are of adult types and appear during adult life in individuals exposed in utero, resembling human experience with diethylstilbestrol. The ENU studies in patas monkeys will provide experimental data indicating whether adult or pediatric tumor types are more likely to develop in at least one species of primate in response to carcinogenic exposure in utero, and thus whether chemical carcinogens are likely to be involved in the genesis of pediatric or adult types of tumors in man.

A new class of chemical carcinogens, the water-soluble organic polycations, have proposed uses in medicine such as inclusion in veterinary vaccines and in interferon-inducing complexes. Furthermore, they are non-reactive chemical carcinogens, differing markedly in this respect from the usual low molecular weight organic compounds, and elucidation of their mechanism of action, particularly resolution of the question of the possible role of oncogenic viruses, may provide a useful tool in the study of combined effects in chemical carcinogenesis, as well as provide a rational basis for their inclusion or exclusion from pharmaceutical use on the basis of carcinogenic activity.

Carcinomas of the gastrointestinal tract and pancreas comprise the largest source of cancer morbidity in the adult population of the United States. Existing "intestine-selective" carcinogens are much less specific than is desirable. The high specificity of methyl(acetoxymethyl)nitrosamine for intestinal mucosa provides a new carcinogen well suited to studies of physiological and biochemical factors significant in development of intestinal carcinoma.

Proposed Course of Project:

Intestinal carcinogenesis by DMN-OAc will be explored further to define the effects of different doses and routes of administration on different strains of rats, mice, and hamsters. The effect of age at exposure on the spectrum and latency of induced tumors will be ascertained, with emphasis on the carcinogenic effects in fetal and neonatal rats. The metabolism and pharmacodynamics of distribution and excretion, especially the role of bile, will be studied using DMN-OAc-^{14}C.

Transplantation studies of mouse and rat neurogenic tumors, and biochemical and ultrastructural characterization of cloned cultured cells derived from these, will be continued with emphasis on (1) medulloblastomas; (2) the establishment of cultures of possible medulloblastoma precursor cells from DMBA-treated rats; (3) identification of differentiated biochemical functions to confirm or exclude the hypothetical neuroectodermal origin of several classes of spontaneous and chemically inducible tumors of rats and mice.

Patas monkeys exposed to ENU either transplacentally or directly (after birth) should continue to be observed for the development of tumors. Pharmacodynamic studies will be initiated to compare maternal-fetal distribution and tissue/organ localization of ENU-^{14}C in this species, versus the rat and mouse. Comparative dose-response curves for ENU-induced teratogenic effects in patas monkeys and Sprague-Dawley rats will be carried out as a rapid measure of relative susceptibility to this compound.

Rat renal "interstitial cell" tumors will continue to be studied in organ culture to determine whether or not the morphologically mesenchymal tumors can be induced to form characteristic renal tubules and glomeruli, thus confirming the relation of such neoplasms to nephroblastomas. Once the model is established, it should be used to determine the effects of manipulations of physiological parameters, including hormonal balance and immunological responsiveness, on the genesis and behavior of these tumors. Studies should also be made to determine what synergisms may exist between carcinogens and other non-oncogenic teratogens, including vitamin A deficiency, in order to determine whether we can duplicate in the experimental rat system, the observed association in man of aniridia and urogenital malformations (known to be inducible in the rat by vitamin A deficiency) with an excess occurrence of Wilms' tumor.

Project No. Z01-CP-04687-05-EXP

Honors and Awards

Visiting Fellow of the Max-Planck-Gesellschaft, Max-Planck-Institut für Hirnforschung, Cologne, Germany; Sept. 11 - Oct. 12, 1974

Publications

Vesselinovitch, S. D., Rao, K. V. N., Mihailovich, N., Rice, J. M., and Lombard, L. S.: Development of broad spectrum of tumors by ethylnitrosourea in mice and the modifying role of age, sex, and strain. Cancer Res. 34: 2530-2538, 1974.

Levy, H. B., Baer, G., Baron, S., Gibbs, C. F., Iadarola, M., London, W., and Rice, J. M.: Induction of interferon in non-human primates by a nuclease-resistant polyriboinosinic polyribocytidylic acid complex. IRCS J. Int. Res. Commun. (Med. Sci.) 2: 1643, 1974.

Lombard, L. S., Rice, J. M., and Vesselinovitch, S. D.: Renal tumors in mice: Light microscopic observations of epithelial tumors induced by ethylnitrosourea. J. Natl. Cancer Inst. 53: 1677-1685, 1974.

Project No. Z01-CP-04505-01-EXP
1. Experimental Pathology Branch
Carcinogenesis, DCCP
2. Tumor Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Comparative Pathology of Neoplasms Caused by Environmental Carcinogens

Previous Serial Number: NCI-4397

Principal Investigator: Carolyn H. Lingeman, M.D.

Other Investigators: None

Cooperating Units: Laboratory of Pathology, NCI; Epidemiology Branch, NCI; Center for Disease Control; National Institute of Occupational Safety and Health; Armed Forces Institute of Pathology; Pathologists of other laboratories in the United States and foreign countries.

Man Years:

Total	: 0.7
Professional	: 0.5
Other	: 0.2

Project Description

Objectives:

To define and compare the epidemiologic, behavioral and morphologic characteristics of malignant neoplasms known or suspected of being caused by carcinogens in the environment.

Methods Employed:

In cooperation with pathologists, epidemiologists and other scientists inside and outside the NCI, histologic materials from humans and animals exposed to known or suspected carcinogens are being examined and compared. Most effort during this year was devoted to vinyl chloride-associated neoplasms but studies of other types of neoplasms suspected of being related to carcinogens in the environment were continued.

Major Findings:

1. Etiology of Cancer of the Ovary:

Epidemiologic and clinical information and histologic material from humans and animals with malignant neoplasms of the ovary were examined and compared in an attempt to identify high risk factors associated with specific histologic types. All evidence to date indicates that cancers of the human ovary are the results of yet-to-be identified carcinogens in the environment acting on genetically or otherwise susceptible hosts. Although a variety of chemical compounds and irradiation can induce neoplasms in ovaries of animals, only asbestos and talc have been seriously considered as candidate carcinogens for the human ovary. Female asbestos workers appear to have a higher-than-expected risk of developing cancer of the ovaries but evidence of an association with asbestos for the majority of girls and women is lacking and exposure to asbestos, talc and other chemical, physical and viral agents are difficult to evaluate. Assessment of risk factors requires attention to the histologic type of neoplasms. There are differences in the epidemologic characteristics and presumably in the etiologies of different types. For example, neoplasms of germ cell origin may be relatively more frequent in individuals of oriental heritage than in caucasians. Black women of the United States and Uganda appear to have a greater risk of developing malignant teratomas. Neoplasms of germ cell origin, stromal cell origin and epithelial neoplasms show characteristic and different age distributions. Within the epithelial group which account for most of the fatal neoplasms in the U. S., the most frequent type, papillary-serous cystadenocarcinomas, tend to occur at younger ages and the shape of the age distribution is similar to that of breast cancer in the middle age group but not at extremes of age. High risk factors for papillary-serous cystadenocarcinomas include family history of similar neoplasms, previous carcinoma of the breast, residence in industrialized countries (except Japan), and possibly nulliparity and infertility, although the associations are not as clear as for breast cancer. A protective effect of a first pregnancy at a young age has not yet been shown to protect against cancer of the ovary as it has for the postmenopausal type of breast cancer. High risk factors for other specific histologic types of neoplasms of the ovary include 3 genetic syndromes: the Peutz-Jeghers, and basal cell syndromes, and some types of gonadal dysgenesis. Neoplasms are infrequent in ovaries of most animals although some types that are relatively infrequent in women can be induced in mice by irradiation and some chemical carcinogens. Chickens of certain flocks develop spontaneous adenocarcinomas that histologically and biologically mimic the frequent cystadenocarcinomas of human ovaries. Studies to evaluate the immediate environment of such chickens are being planned.

2. Occupational Carcinogenesis: The Pathologic Effects of Exposure to Vinyl Chloride (VC): Reports early in 1974 indicated that a rare type of malignant neoplasm of the liver (angiosarcoma) was associated with occupational exposure to VC gas. This provided an unusual opportunity to study the effects of a toxic and carcinogenic chemical compound on man and animals (knowledge of the carcinogenicity of VC in animals dates back to 1971 or earlier). In a cooperative

study coordinated by officials of NIOSH and CDC, histologic materials were taken from (1) human VC polymerization workers with and without angiosarcomas, (2) humans with angiocarcomas of the liver without known exposure to VC, and (3) workers in VC industrial plants not involved directly in the polymerization process. The materials were assembled in the Laboratory of Pathology, NCI, and examined by pathologists with special interests and experience in cancer and diseases of the liver (the Institute was fortunate to have the participation of Dr. Hans Popper who was in residence at NIH as a Fogarty Scholar when the study began). The angiosarcomas and associated pathologic changes of VC workers are being compared to (1) those of individuals exposed to thorotrast and arsenic (carcinogens known to cause angiosarcomas of the human liver) (2) to those without known etiologies and (3) to those of animals exposed to VC, other carcinogens and some that are "spontaneous." Although the studies are incomplete, preliminary findings include: Angiosarcomas and associated pathologic effect of VC -- Angiosarcomas in the liver liver and other organs of VC polymerization workers are remarkably similar to those induced in experimental animals by inhalation of VC. Human and animal angiosarcomas are composed of sinusoid-lining cells that proliferate in sinusoids, compressing, isolating and eventually replacing hepatocytes. They form large blood-filled cavernous spaces and are frequently multicentric. Whether similar neoplasms observed in other organs (lung, heart, kidney etc.) are metastases or primary neoplasms remains to be determined. The livers of VC polymerization workers, whether or not they contain angiosarcomas, show a characteristic type of fibrosis, a change that is also observed in experimental animals although to a lesser extent.

Louisville Mortality Study -- Medical records and histologic material from 55 deceased former employees with a variety of occupations from a plant in the United States that engages in polymerization of vinyl chloride and other industrial processes, are being examined for evidence of neoplasms of other types than angiosarcomas or other pathologic effects that might be linked to varying degrees of exposure to vinyl chloride or other chemical compound. Autopsy material was available for 23. These workers, all males except one, were employed in a variety of occupations including a cook, a janitor, a chemist and other miscellaneous jobs that involved varying degrees of exposure to VC and other chemicals. They died of a variety of causes including neoplasms of several sites other than the liver, of cardiovascular disease and miscellaneous causes. These are being evaluated by epidemiologic methods to determine whether excesses of any cell type of neoplasm or other pathologic change could be attributed to exposure to vinyl chloride. None of these individuals had angiosarcomas or the type of hepatic fibrosis observed in VC polymerization workers.

National Hepatic Angiosarcoma Case Finding Survey -- Angiosarcomas of liver and other organs are infrequent in man and fewer than 30 are estimated to occur each year in the U. S. They occur many years following exposure to thorotrast and are though to follow prolonged exposure to inorganic arsenic. At least 20 cases have been observed in vinyl chloride polymerization workers in the United States, Canada, Britain and other countries of Europe. Human angiosarcomas of known

and unknown etiology are being systematically compared to determine whether any specific histologic characteristics can be attributed to vinyl chloride or other carcinogen, but these studies are incomplete.

Epidemiologists of the Center for Disease Control (CDC) have located and made available for study histologic material from more than 100 cases of hepatic angiosarcomas without known exposure to VC diagnosed in the United States during the 11 year period 1964-1974 by means of letters to pathologists and inquires made to tumor registries. Several of these patients have histories of exposure to thorotrast (known to be associated with angiosarcomas) and arsenic (strongly suspected of causing hepatic angiosarcomas). In addition, histologic material from livers of 3 Portuguese patients who died of thorotrast-associated angiosarcomas were made available. Officials of the Connecticut Tumor Registry revealed that 8 angiosarcomas of the liver were reported to the Registry between 1935-1973. Examinations from biopsy and/or autopsy material confirmed a diagnosis of hepatic angiosarcomas in 6 of these, 2 of whom were workers in fabrication plants utilizing vinyl chloride polymers and 2 others lived in vicinities of the 2 fabrication plants. The latter observation is important in the implication that VC or other carcinogenic compounds processed in certain industrial plants may be entering the immediate environments of the plants in dangerous concentrations.

Comparative Pathology of Angiosarcomas of Animals -- Histologic material from untreated dogs with angiosarcomas of liver and other sites was obtained from the Epidemiology Branch of the NCI and pathologists of the AFIP. Histologic material from untreated mice bearing the hairless gene and mice of other strains treated with vinyl chloride and other chemical carcinogens known to cause angiosarcomas of liver and other organs from the NCI Registry of Experimental Cancers and other laboratories is being examined and neoplasms are being compared to those of dogs and man. Findings to date include the facts that angiosarcomas of liver, heart, lungs and other organs are not uncommon in dogs, particularly German shepherds in military service, in untreated mice bearing the hairless gene and in mice of other strains receiving a variety of chemical compounds including vinyl chloride, urethane and o-aminoazotoluene. Vinyl chloride also causes angiosarcomas in rats and hamsters, and neoplasms of other cell types in rats including Zymbal gland carcinomas and nephroblastomas. Although studies comparing histologic features of angiosarcomas in man and animals is only in preliminary phases, all evidence to date reveals remarkable similarities regardless of species or inducing agent.

Significance to Biomedical Research and the Program of the Institute:

Despite evidence that most human cancers are caused by carcinogens in the environment, few specific chemical or physical agents are "proven" carcinogens for man. Employees of industrial plants offer unusual opportunities in cancer detection and prevention because duration and intensity of exposure to known or suspected carcinogens can be readily documented and tested in animals unlike the complex and uncertain exposures of the population at large

in which effects of suspected carcinogens are difficult to measure and separate and to relate to onset of a specific type of cancer. Pathologic definition is important in assessment of neoplasms known to be associated with occupational exposure to carcinogens as well as those without such associations. The latter group constitutes the majority of cancers considered to be caused by factors in the environment and the differences in histologic characteristics correlated with epidemiologic features can suggest etiologic mechanisms to be investigated.

Proposed Course of Project:

In cooperation with officials of other government agencies (NIOSH, OSHA, EPA, FDA, CDC), and pathologists and within and outside the NCI, to continue to study and compare morphologic changes in tissues of humans and animals with documented exposure to industrial carcinogens (known and suspected). Such high risk populations offer opportunities to develop realistic methods for prevention and detection of cancers and identification of precancerous changes.

Honors and Awards

Invited participant "Ovarian Carcinoma Symposium," Reston, Va., Nov. 15-16, 1974.

Invited Speaker on health effects of vinyl chloride presented to Interagency Collaborative Group on Environmental Carcinogenesis, Dec. 17, 1974.

Publications

Lingeman, C. H.: Etiology of cancer of the human ovary: A review. J. Nat. Cancer Inst. 53: 1603-1618, 1974

Project No. Z01-CP-04512-01-EXP
1. Experimental Pathology Branch
Carcinogenesis, DCCP
2. Tumor Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Morphogenesis of Pancreatic Adenocarcinoma

Previous Serial Number: None

Principal Investigator: Morton H. Levitt, M.D.

Other Investigators: Curtis Harris, M.D., Robert Squire, D.V.M., Ph.D., Stephen Springer, Ph.D.*, and Martin Wenk, Ph.D.*

Cooperating Units: *Microbiological Associates, Inc.

Man Years:

Total	: 0.6
Professional	: 0.5
Other	: 0.1

Project Description

Objectives:

To delineate the morphogenesis of pancreatic carcinoma induced by 2,2'-dihydroxy-di-N-propylnitrosamine (DIPN) through use of high resolution light microscopy, transmission electron microscopy, and quantitative microscopic autoradiography.

Methods Employed:

Random-bred male Syrian golden hamsters, beginning at 8 weeks of age received DIPN subcutaneously in a dose of 250 mg/kg body weight once weekly, in olive oil, with an appropriate control group. A second paired experiment was initiated substituting distilled water for the olive oil vehicle.

Major Findings:

Since this project was initiated in the late spring, no major findings can be reported at this time.

Significance to Biomedical Research and the Program of the Institute:

Pancreatic cancer is an increasingly common neoplasm affecting man, ranking fifth in overall incidence and fourth as a cause of cancer mortality in the United States. The emergence of an experimental animal model for the induction and study of pancreatic neoplasms is very new. This project seeks to confirm this new model and delineate the morphogenesis of this lesion.

Proposed Course of Project:

Groups of 60 test and 20 control animals will be sacrificed at 12 time points over a 40 week period after receiving an IP injection of ^{3}H-Thymidine one hour prior to sacrifice. Sections of pancreas will be fixed and embedded for examination by high resolution light microscopy and transmission electron microscopy as well as quantitative light microscopic autoradiography.

A second experiment will seek to reproduce the findings of Pour et al. as reported in the Am. J. Pathol. 76: 349-358, 1974, who obtained a high incidence of pancreatic neoplasms in Syrian golden hamsters following subcutaneous injection of DIPN weekly for life, with a tumor latency as short as 15 weeks. Paired groups of 40 test and 20 control animals will be used in a lifetime study, employing both olive oil and water as the vehicle. Twelve selected tissues will be examined histologically following necropsy.

A third experiment will seek to examine the uptake and localization of the carcinogen in the pancreas by means of injection of ^{3}H-DIPN and serial sacrifice at 5 time points over a 72 hour period in a group of 20 test and 5 control animals, utilizing water as the vehicle.

Project No. ZO1-CP-04514-01-EXP
1. Experimental Pathology Branch
Carcinogenesis, DCCP
2. Tumor Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Development of Pathology Reference Standards in Carcinogenesis Studies

Previous Serial Number: None

Principal Investigator: Robert A. Squire, D.V.M., Ph.D.

Other Investigators: None

Cooperating Units:

Man Years:

Total	: 1.0
Professional	: 0.5
Other	: 0.5

Project Description

Objectives:

1) Develop a standardized pathology protocol to assure uniform and thorough pathologic evaluation of animals under test in carcinogenesis screening studies, specifically those in the Bioassay Segment Programs.

2) To arrive at a consensus for nomenclature and definitions of specific, controversial rat liver lesions which are frequent end-points in carcinogenesis studies.

Methods Employed:

Following consultation with several pathologists and other scientists, a protocol was prepared which details the minimum level of gross and microscopic examination of animals on test. It also provides guidelines for determining necessary facilities, technical methods, and personnel qualifications.

To establish definitions of rat liver lesions, twenty pathologists with wide and varied experience in laboratory animal pathology and experimental carcinogenesis were convened for a 3 day workshop. Slide sets representing several specific, controversial lesions were distributed in advance and were projected

and discussed at the workshop. After considerable debate and consideration of all available relevant experimental and biologic information, a nomenclature was selected by majority vote. Whenever possible, the biologic nature of the lesion, as viewed by the participants, was reflected in the nomenclature.

Major Findings:

1) The standardized protocol that was developed defines methods for gross necropsy and histologic examination including specific tissues which are to be examined in test and control animals. Complete gross necropsies of all animals are required. Histologic examination of all control animals, chronic test animals, and selected animals on dose finding studies are also required. The histologic examination includes 27 standard tissues plus gross lesions and tissue masses. Qualifications of personnel and facilities are defined and methods of recording and reporting pathologic findings are described.

2) As a result of the rat liver workshop, it was recommended that the lesions in the study set generally termed "hyperplastic nodules" be designated as neoplastic nodules. This reflected the participants interpretation of all available evidence, which indicates that these lesions are neoplastic rather than hyperplastic according to the traditional definitions of the terms. They are part of the spectrum of liver carcinogenesis and indicate, at a minimum, an increased probability for the development of hepatocellular carcinoma.

Criteria for the diagnosis of hepatocellular carcinoma in this specific study set were also developed.

Significance to Biomedical Research and the Program of the Institute:

Pathologic evaluations are critical end-points in carcinogenesis studies, and standardized, thorough methods are required to reach accurate conclusions. The Carcinogenesis Program has a scientific responsibility for detecting chemical and physical carcinogens in the environment, and adherence to an appropriate pathology protocol will contribute to the accurate appraisal and analysis of in vivo bioassay studies.

Many carcinogenesis studies in rats have resulted in the induction of hepatic nodules and cancers, and use of the term "hyperplastic nodule" has been misleading. The term neoplastic nodule will indicate that these lesions are considered to be stages in the development of cancer, which will have significant impact upon the interpretation of rat carcinogenesis studies in the Carcinogenesis Program and on an international scale.

Proposed Course:

The protocol will continue to be followed, and periodic evaluations will be made to determine if revisions are necessary to meet the needs of the Program, and future workshops will be organized to address other controversial areas in carcinogenesis pathology.

Project No. ZO1-CP-04548-03-EXP
1. Experimental Pathology Branch
Carcinogenesis, DCCP
2. Tumor Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Registry of Experimental Cancers

Previous Serial Number: Same

Principal Investigators: Dr. Harold L. Stewart, Consultant
Dr. Thelma B. Dunn, Consultant
Dr. Margaret D. Barrett (Dr. Margaret K. Deringer)

Other Investigators: None

Cooperating Unites: None

Man Years:

Total	: 4
Professional	: 2
Other	: 2

Project Description

Objectives:

(1) The storage and retrieval of pathological material and data on cancers and other lesions of laboratory animals (primarily rodents), and (2) the use of such for research and educational purposes.

Methods Employed:

The methods employed in the work of the Registry involve the selection of protocols, pathologic material, including histologic slides, paraffin blocks, and gross specimens; and illustrations in the form of lantern slides, gross photographs and photomicrographs in black and white and in color. The work of the Registry also includes the collection of records of experiments, reprints and references on this material. The Registry of Experimental Cancers possesses a large collection of spontaneous and induced cancers and other lesions. The pertinent information on the collection is being indexed. Many of the data have been prepared for and entered into the computer.

The Registry accesses material from investigators at NCI, other Institutes of NIH, other Governmental agencies, industrial laboratories, and universities here and abroad. A total of 408 single or group accessions from investigators outside of NCI have been processed since March 1971. The Registry is preparing Study Sets of slides, with explanatory material, relating to particular areas of the pathology of cancer in rodents. Sets of neoplasms of the lung ("Tumors and Nonneoplastic Proliferative Lesions of the Lungs of Mice") have been completed. These sets will be loaned to interested investigators in this country and abroad.

The Registry has Study Sets of slides on "Hematopoietic and Lymphoreticular Neoplasms" (prepared for a workshop on the comparative pathology of these neoplasms at NCI in May 1971) and on "Induced and Spontaneous Tumors of Small Intestine and Peritoneum in Mice", which are loaned, with descriptive material, to investigators who request them. Eight loans for periods up to two months have been made.

Nine investigators have spent varying periods of time at the Registry studying some of the available material.

Investigators come to the Registry for consultation. There have been single or multiple consultations with 77 individuals since February 1974.

Major Findings:

The functions (outlined in Objectives) of the Registry in the wider field of cancer research and more particularly in the Carcinogenesis program are such that there are no major findings to report.

Significance to Biomedical Research and the Program of the Institute:

The availability of the wealth of material possessed by the Registry advances the knowledge of spontaneous and induced disease processes in animals.

The existence of the Registry will contribute to the standardization of nomenclature of cancers and other lesions in laboratory rodents. Slides and protocols from the Registry are used to illustrate and describe lesions discussed at the weekly slide conferences.

The members of the Registry serve as consultants in the monitoring of pathology from laboratories under the Bioassay Operations contracts.

Proposed Course of Project:

The Registry will continue and expand all of its activities (already set forth in this report).

Consultation between the Registry and the European Late Effects Project Group, Mol/Donk, Belgium and the International Agency for Research on Cancer, Lyon, France will continue.

Project No. Z01-CP-04548-03-EXP

Publications

Stewart, H.L., Deringer, M.K., Dunn, T.B. and Snell, K.C.: Malignant schwannomas of nerve roots, uterus, and epididymis in mice. J. Natl. Cancer Inst. 53:1749-1758, 1974.

Dunham, L.J., Snell, K.C. and Stewart, H.L.: Brief communication: Argyrophilic carcinoids in two Syrian hamsters (Mesocricetus auratus). J. Natl. Cancer Inst. 54:507-513, 1975.

Stewart, H.L., Dunn, T.B., Snell, K.C. and Deringer, M.K.: Tumors and proliferative lesions of the respiratory apparatus in mice. In Turusov, V.S. (Ed.): Pathology of Tumours in Laboratory Animals. In press.

Stewart, H.L.: Tumours of the soft tissues. In Turusov, V.S. (Ed.): Pathology of Tumours in Laboratory Animals Vol. II Tumours of the Mouse. In press.

Stewart, H.L. and Snell, K.C.: Patterns of neoplastic and nonneoplastic diseases of Praomys (Mastomys) natalensis. In: Recent Results in Cancer Research. In press.

Stewart, H.L.: Comparison of sites of multiple neoplastic and atrophic lesions induced by carcinogenic hydrocarbons and fluorenylamine compounds. In Severi, L. (Ed.): Multiple Primary Malignant Tumours. In press.

Deringer, M.K.: Multiple primary tumors in breeding females of four strains and three substains of inbred mice. In Severi, L. (Ed.): Multiple Primary Malignant Tumours. In press

Stewart, H.L.: Comparative aspects of certain cancers. In Becker, F.F. (Ed.): Cancer A Comprehensive Treatise. In press.

SUMMARY REPORT

LUNG CANCER BRANCH

July 1, 1974 through June 30, 1975

"Plans, develops and implements a research program on the pathogenesis and prevention of lung cancer involving (1) identification and study of agents and mechanisms involved in the induction and development of lung cancer in man; (2) correlation of findings in human and animal studies in order to define relevance of animal models for study of human lung cancer; and (3) identification and study of agents and mechanisms capable of preventing the development of lung cancer. All these studies are pursued in the whole organism, in isolated cellular systems involving organ and tissue culture, and at the molecular level, in animals and man."

Current studies in the Lung Cancer Branch include investigations on the carcinogenic effects of different chemicals on the respiratory epithelium in the whole animal, the use of short-term and long-term organ culture systems to study the effects of carcinogens and anti-carcinogens on respiratory epithelium, investigation of the molecular mechanism of action of lung carcinogens in cell-free systems, and investigation of the molecular mechanism of action of the anti-carcinogenic substance, vitamin A. Studies are pursued within the Branch in a manner that allows correlation of findings at the molecular, cellular, and whole animal level. The intramural program of the Lung Cancer Branch was developed along high priority program lines and is therefore closely coordinated with the contract program of the Lung Cancer Segment. Members of the staff provide scientific direction to the Lung Cancer Segment; in addition, they contribute to the review and monitoring of some contracts in other collaborative segments. In this way, new information obtained at the basic level is rapidly disseminated and utilized in order to apply research results where they will ultimately be of most direct human benefit.

The following is a summary of the investigations within the Branch:
Lung Cancer Pathogenesis Section - *"(1) Develops and evaluates animal models for study of induction and prevention of lung cancer, and the role of various chemicals in inducing or preventing lung cancer. Studies involve the administration of carcinogenic and anti-carcinogenic agents to experimental animals and the evaluation of their effects on the development of lung cancer and (2) develops and evaluates organ culture and tissue culture methods for study of induction and prevention of lung cancer. Studies measure the effects of carcinogenic and anti-carcinogenic agents on the cell population which is directly involved in the development of lung cancer."*

(1) Animal Studies of Respiratory Carcinogenesis - Studies have been continued on the development of squamous cell carcinoma of the respiratory tract of the hamster, using both benzo[a]pyrene (BP) and N-nitroso-N-methylurea (NMU) as carcinogens. It has been previously found that NMU can markedly potentiate the carcinogenic effects of BP on respiratory epithelium, particularly in terms of shortening the latency period for tumor development. These studies contribute to define maximal response conditions for induction of respiratory tumors in the experimental animal. Within the past year, the significant finding has been made that administration of retinyl acetate (given both during and after the period of BP and NMU dosing) markedly reduces or delays the observed respiratory tract tumor incidence.

Examination of histogenesis of respiratory tract tumors in experimental animals suggests that for squamous cell carcinomas of laryngo-tracheobronchial epithelium a number of stages can be identified in the transition to malignant lesions (at the light microscopic level). These stages include phases of squamous metaplasia, dysplasia with focal basal cell hyperplasia, nodular overgrowth and downgrowth basal cells, in situ carcinoma, and finally invasive squamous cell carcinoma. Similar stages of histogenesis of respiratory squamous cell carcinoma have been postulated for development of this common form of cancer in man. The above studies thus further confirm the usefulness and relevance of the animal models that have been developed.

(2) Organ Culture Studies on the Isolated Tracheobronchial Epithelium of Hamster and Man - Excellent progress has been made in this area during the past 12 months. Extensive use is now being made of the system which has been developed in the Lung Cancer Branch for long term organ culture of both hamster and human trachea and bronchi. A chemically defined medium, supplemented with insulin and hydrocortisone, is being used to give excellent long-term in vitro survival of both hamster and human tracheobronchial epithelium, for 2 weeks or more. Using this system, it has been demonstrated that numerous synthetic analogs of vitamin A can reverse keratinizing, squamous metaplastic lesions of hamster tracheal epithelium. In addition to the further confirmation of previous findings that analogs with modifications in the ring portion of the molecule are highly active biologically, it has very recently been found that the polar, terminal portion of the molecule can also be modified with substantial retention of activity. These new synthetic molecules include ether and amine derivatives of vitamin A, which appear to be substantially less toxic than natural forms of vitamin A. The reduction in toxicity offers major advantages in terms of development of a synthetic analog which would eventually be useful for prevention of epithelial cancer in man. The scientific collaboration of Hoffmann-La Roche Inc. has been invaluable in the performance of the above studies.

The organ culture methodology has also proved to be useful for studying the effects of asbestos on respiratory epithelium. Asbestos is a significant co-carcinogen or promoter for development of lung cancer in man; its mechanism of action is almost totally unknown. Studies performed in the Branch during the past year have shown that addition of asbestos to hamster tracheal organ cultures causes a definite epithelial hyperplastic response, which may, in part, explain some of the biological effects seen in man. Efforts are being directed toward inhibiting this hyperplastic response, with the goal of inhibiting the co-carcinogenic or promoting effects of asbestos.

The organ culture methodology described above is now also being extensively used to study human bronchial tissues. Human material has been found to be well maintained in vitro, using electron microscopic and autoradiographic techniques to monitor the cultures. It has been found that human bronchial epithelium can activate benzo[a]pyrene and several other carcinogenic aromatic hydrocarbons to forms that are tightly bound to macromolecules, including DNA. Moreover it has recently been shown that the inhibitor of the microsomal drug-metabolizing system, 7,8-benzoflavone, and the anti-oxidant, butylated hydroxytoluene, both block the binding of benzo[a]pyrene to DNA in cultured human bronchi. The biological significance of lesions induced by carcinogenic hydrocarbons in cultured human bronchial epithelium is being further evaluated in transplantation studies, in which these human bronchi are implanted into immune-deficient nude mice.

(3) Studies on Cell Biology of Other Epithelia - Epidermis and bladder epithelial cell systems may be used as screening models for the response of respiratory epithelium to carcinogens or anti-carcinogens. A small level of effort in the Lung Cancer Branch is being devoted to such research. In particular, an epidermal cell culture system is being used to assay biological activity of new vitamin A analogs. Significant data can be obtained in less than a week. Such cultures employ chemically defined medium, without serum. Addition of retinyl acetate to these cultures causes a significant increase in cellular RNA content. Within the past year, several new analogs of vitamin A have been found to be extremely active in this epidermal cell culture system. The activity of analogs, as measured in the epidermal cell culture system, correlates well with their activity in the tracheal organ culture system. These tissue culture studies thus serve as a primary screen for evaluation of biological activity of new synthetic vitamin A analogs, as well as being useful for further elucidation of the mechanisms of action of these compounds. Since the eventual use of vitamin A analogs for cancer prevention in man is currently under consideration, it is of utmost importance to have the most efficient primary screen for assessment of activity.

Since bladder epithelium shows many biological similarities to respiratory epithelium (including a relatively high incidence of bladder cancer in people who smoke heavily), some new studies have been started during the past year on organ culture of bladder epithelium. Optimal culture conditions for bladder have been defined, and a comparison of the response of respiratory and bladder epithelium to carcinogens, as well as to vitamin A analogs, is being made.

Differentiation Control Section - *"Studies molecular processes involved in control of cellular differentiation in respiratory epithelium as related to the induction and prevention of lung cancer. Particular emphasis is placed on the biochemical mechanisms whereby selected compounds inhibit or prevent the development of lung cancer."*

Since vitamin A is a required substance for normal differentiation of respiratory epithelium, and since it inhibits the effects of carcinogenic substances such as benzo[a]pyrene, both in vivo and in vitro, studies on the

mechanism of action of this substance are being actively pursued. During the past year, several advances in this area have been made. A short term culture of intestinal mucosal cells for studies on the biosynthesis of glycophospholipids with vitamin A as a structural component has been developed. Studies on nutritional requirements for vitamin A of different cell types and differentiated states have been performed A comparison of the content of retinol and retinyl esters in bronchogenic squamous cell carcinoma and intestinal adenocarcinoma showed that the squamous cell carcinoma has no detectable retinol or retinyl-esters, in contrast to the adenocarcinoma, which has as much retinol and esters as normal intestinal cells. Retinyl phosphate has been chemically synthesized for the first time. When this compound is incubated in the presence of epithelial membranes (liver, intestine), it accepts monosaccharides, destined for membrane assembly. New gas liquid chromatography systems have been developed for detection of as little as 0.2 microgram of vitamin A. In other studies, mouse epidermal cells, when cultured in the presence of retinyl-acetate, have been found to acquire new synthetic properties, with a 5 to 10 fold increase in the biosynthesis of membrane and secreted glycoproteins. In many of the above studies, the effects of vitamin A appear to be highly specific for particular glycoprotein or glycopeptide fractions. The above studies thus provide an insight into the fundamental role of vitamin A in determining structure and function of particular proteins, especially those of the cell membrane.

Project No. Z01-CP-04798-05-LC
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Differentiation Control Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Role of Vitamin A in Control of Epithelial Cell Differentiation and Carcinogenesis

Previous Serial Number: Same

Principal Investigators: Luigi M. De Luca, Ph.D., Jacques Frot-Coutaz, Ph.D., Carol S. Jones, M.S.

Other Investigators: Stuart H. Yuspa, M.D., George Wolf, Ph.D., P. R. Sundaresan, Ph.D., Paul Nettesheim, M.D., Jerrold Ward, D.V.M., Ph.D.

Cooperating Units: Experimental Pathology Branch, NCI; Massachusetts Institute of Technology, Cambridge, Massachusetts; Laboratory of Lipid Research, St. Joseph Hospital, Lancaster, Pennsylvania; Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Man Years:

Total	3.00
Professional	2.00
Other	1.00

Project Description

Objectives:

To study control mechanisms of Mucous-squamous cell differentiation and membrane growth by vitamin A and chemical carcinogens.

Methods Employed:

The mode of action of vitamin A is studied in the following biological systems:

a. Chemically induced transplanted squamous cell bronchogenic carcinoma of the rat, obtained from Dr. Paul Nettesheim, Oak Ridge National Laboratory; and chemically induced transplanted intestinal adenocarcinoma of the rat, obtained from Dr. Jerrold Ward, Carcinogen Metabolism and Toxicology Branch.

b. Short term culture of intestinal mucosal cells from the rat and the hamster.

c. Epidermal cell culture in the presence and absence of retinyl-acetate.

d. In Vivo studies on liver emembranes of normal, vitamin A deficient and vitamin A excess treated hamsters and rats.

Biochemical Methods

a. Retinyl-phosphate and derivatives. The involvement of retinol as a structural component of the membrane in the following series of reactions has been studied:

(1) Retinol + ATP $\rightleftarrows$ Retinyl - PO_4 + ADP

(2) Retinyl-PO_4 + glycosyl-nucleotide $\rightleftarrows$ Retinyl-phosphate-glycose + nucleoside -diphosphate

(3) Retinyl-phosphate-glycose + Acceptor $\rightleftarrows$ Retinyl-phosphate + glycosyl-acceptor.

Reactions (1) and (2) have been studied in vitro in a liver membrane system. Isolation of phosphorylated retinol derivatives has been achieved by chromatography on columns of DEAE-cellulose and silicic acid. In addition, preparative chromatography on layers of silica gel G allows separation of this family of compounds from compounds of longer polyprenol chain length, like the naturally occurring dolichols and their phosphorylated derivatives. However separation of the possible different glycosyl derivatives of retinyl-phosphate has not been achieved, as yet. A start in applying high pressure liquid chromatography to this problem has been made.

The chemical synthesis of retinyl-phosphate has made possible to demonstrate that this molecule is an intermediate to retinyl-phosphate-glycose. Most of these studies have been conducted in vitro, in isolated membranes.

Whole Cell studies were deemed necessary to demonstrate the validity of the in vitro cell-free studies. [^{14}-] retinol is routinely used in a short term culture of intestinal cells from hamsters or rats. Kinetic studies have demonstrated the enzymatic character of the incorporation. The retinol phospholipidic compounds are extracted with chloroform (2):methanol(1). Their nature and similarity to or dissimilarity from in vitro membrane synthesized compounds is being studied. A major methodological improvement has been the analysis of retinol and similar compounds, like retinoic acid and anhydroretinol, by gas liquid chromatography and scintillation counting. The glycosidic component of retinyl-phosphate-glycose is analyzed after hydrolysis, reduction to hexitols and gas liquid chromatography of the boronic acid derivatives of the hexitols.

Major Findings:

a. Study of the relationship between the content of vitamin A and the state of differentiation of epithelial tissues. Since keratinizing epithelial arise focally in vitamin A deficient mucous epithelia, we asked whether keratinizing tissues, like the bronchogenic carcinoma, and mucous secreting tissues varied greatly in the amount of retinol and retinyl-esters they contained. Normal intestinal mucosa was also studied. Animals were fed laboratory chow and the tumors were transplanted and maintained their keratinized or mucus secreting phenotype. Recovery of retinol and retinyl-esters was checked with a minute amount of radioactive tracer. It was found that bronchogenic squamous cell cacrcinoma has no detectable retinol or retinyl esters. On the contrary, intestinal adenocarcinoma has about the same amount of retinol and retinyl esters as normal intestinal mucosa. These studies were conducted in collaboration with Drs. P. R. Sundaresan, P. Nettesheim and J. Ward.

b. Studies on the biosynthesis of retinyl-phosphate and derivatives in short term culture of intestinal cells. It was found that intestinal mucosal cells in culture are very active in synthesizing a compound with chromatographic behavior similar to chemically synthesized retinyl-phosphate.

c. Studies on the biosynthesis of glycoproteins by epidermal cells, cultured in the presence and absence of retinyl-acetate.

Two sets of labelled precursors were used: 1) [^{3}-H]-fucose and [^{14}-C]-glucosamine; and 2) [^{3}-H]-galactose and [^{14}-C]-mannose. Labelled glycoproteins were obtained from normal cells and cells grown in the presence of retinyl-acetate. It was found that vitamin A stimulated the incorporation of all precursors from two to ten fold. These studies are conducted in collaboration with Dr. Stuart Yuspa.

d. Studies on the biosynthesis of membrane glycolipids and glycoproteins in normal, vitamin A deficient and vitamin A excess treated hamster liver.

These studies were conducted with [^{14}-C]-mannose as a precursor. It was found that even at a very early stage of vitamin A deficiency, before weight loss occurred, the biosynthesis of membrane glycolipids and glycoproteins is reduced to 20% or less or normal membranes. Determination of pool sizes of [^{14}-C] mannose has shown similar values for normal and vitamin A deficient livers. The specific radioactivity for this pool of mannose was greater in vitamin A deficiency, thus excluding the possibility that the observed severe decrease in glycoprotein and glycolipid synthesis is a reflection of a similar decrease in specific radioactivity of the precursor pool. Another important finding was that the amount of covalently bound mannose was 78% less in vitamin A deficiency than in normal conditions. These studies support the view that retinol is involved in the biosynthesis of the membrane.

Project No. Z01-CP-04798-05-LC

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the identification of key control mechanisms in the epithelial cell differentiation; it is a part of the lung cancer program addressed to the development of mechanisms of inhibition and control of respiratory carcinogenesis. The control of mucus cell differentiation by vitamin A is also of great interest in helping to understand the mechanism of carcinogenesis in epithelial tissues in general. The vitamin controls goblet cell formation and its absence leads to hyperplasia of basal cells, squamous metaplasia and keratinization: a sequence of events similar to those taking place in the respiratory epithelium after exposure to benzopyrene. The fucose-glycopeptide, shown to be present in the mucous structures of most epithelia can be used as a specific marker for differentation responses in epithelial tissues.

Proposed Course of Project:

(1) To determine the structure of the vitamin A mannophospholipid by NMR and MS. (2) To study its specific role as precursor of membrane glycoproteins. (3) To study possible alterations of vitamin A dependent mechanisms of membrane growth in solid tumors and in transforming tissues.

Honors and Awards

Visiting Lecturer, Massachusetts Institute of Technology, Cambridge, Massachusetts June 30, 1974 thru July 1975.

Lectures on vitamin A at following Departments:

October 9, 1974 - Department of Biochemistry, Royal Free Hospital School of Medicine, London, England.

October 11, 1974 - Department of Biochemistry, The University of Liverpool, Liverpool, England

October 16, 1974 - Deutches Krebsforschrangszentrum, Institute of Biochemistry, Heidelberg, Germany.

October 22, 1974 - Polish Academy of Sciences, Institute of Hematology, Warsaw, Poland

February 26, 1975 - Oak Ridge National Laboratory, Oak Ridge, Tennessee

Publications

Bonanni, F., and De Luca, L. M.: Vitamin A-dependent fucose-glycopeptide from rat tracheal epithelium. Biochim. Biophys. Acta 343, 632-637, 1974.

De Luca, L.M., and Yuspa, S.H.: Altered glycoprotein synthesis in mouse epidermal cells treated with retinyl-acetate in vitro. Exp. Cell Res. 86: 106-110, 1974.

Barr, R.M., and De Luca, L.M.: The *in vivo* incorporation of mannose, retinol and mevalonic acid into phospholipids of hamster liver. Biochem. Biophys. Res. Commun. 60: 355-363, 1974.

Rosso, G., De Luca, L.M., Warren, C., and Wolf, G.: Enzymatic synthesis of mannosyl retinyl phosphate from retinyl phosphate and guanosine diphosphate mannose. J. Lipid Res., in press.

Project No. Z01-CP-04549-03-LC
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Organ Culture Studies on Tracheobronchial Epithelium

Previous Serial Number: Same

Principal Investigators: Michael B. Sporn, M.D., David Reese, Ph.D., Dianne Newton, Nancy Dunlop, M.S., and Joseph M. Smith

Other Investigators: None

Cooperating Units: None

Man Years:

Total	3.1
Professional	1.1
Other	2.0

Project Description

Objectives:

The principal objective is to study the direct effects of carcinogens and anti-carcinogens on the cell population which is of immediate relevance for development of the most common form of human lung cancer, namely bronchogenic carcinoma.

Methods Employed:

Classical methods of organ culture are being used. Tracheas are being obtained from either normal animals, from vitamin A-deficient animals, or from animals treated wtih respiratory carcinogens.

Major Findings:

The optimal conditions for long-term organ culture of tracheobronchial epithelium of vitamin A-deficient hamsters have previously been defined, using medium CMRL-1066. It has now been found that tracheobronchial epithelium from hamsters treated with respiratory carcinogens such as benzo[a]pyrene or nitrosomethylurea can be organ cultured successfully using

Ham's F-12 medium. The effects of numerous synthetic analogs of vitamin A, in terms of their ability to reverse keratinizing squamous metaplasia of tracheal epithelium, have been evaluated during the past year.

Particularly active analogs are 13-cis-retinoic acid, the dimethylacetyl-cyclopentenyl analog of retinoic acid, and the trimethylmethoxyphenyl analog of retinoic acid. More recently, retinyl methyl ether and retinyl-N-acetyl-amine have also been found to be highly active compounds. Saturation of the double bonds in the side chain of the vitamin A molecule has been found to cause marked loss of activity.

Significance to Biomedical Research and the Program of the Institute:

The finding that new analogs of retinyl acetate, with modifications in the ring portion of the molecule, are directly active on the tracheal epithelium is particularly important. This finding opens the way for synthesis of new forms of vitamin A that may have greater pharmacological usefulness in man for prevention of lung cancer. Studies are currently in progress in the Lung Cancer Branch to evaluate the direct effects of retinyl acetate and its analogs on preneoplastic respiratory epithelium of hamsters, in order to determine if the progression of these lesions can be arrested or reversed in organ culture.

Proposed Course of Project:

To continue as outlined above.

Publications

Clamon, G.H., Sporn, M.B., Smith, J.M., and Henderson, W.R. Effect of α-retinyl acetate on growth of hamsters fed vitamin A-deficient diet. J. Nutr. 105: 215-219, 1975.

Sporn, M.B., Clamon, G.H., Dunlop, N.M., Newton, D.L., Smith, J.M., and Saffiotti, U.: Activity of vitamin A analogues in cell cultures of mouse epidermis and organ cultures of hamster trachea. Nature 253: 47-50, 1975.

Clamon, G.H., Sporn, M.B., Smith, J.M., and Saffiotti, U.: α- and β-retinyl acetate reverse metaplasias of vitamin A deficiency in hamster trachea in organ culture. Nature 250: 64-66, 1974.

Sporn, M.B., Clamon, G.H., Smith, J.M., Dunlop, N.M., Newton, D.L., and Saffiotti, U.: The reversal of keratinized squamous metaplastic lesions of vitamin A deficiency in tracheobronchial epithelium by vitamin A and Vitamin A analogs in organ culture: A model system for anti-carcinogenesis studies. In Karbe, E. and Park, J. (Eds.): Experimental Lung Cancer, Heidelberg, Springer-Verlag, 1974, pp. 575-582.

Project No. Z01-CP-04551-03-LC
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Eukaryotic DNA Synthesis and Carcinogenesis

Previous Serial Number: Same

Principal Investigator: David G. Kaufman, M.D., Ph.D.

Other Investigators: William K. Kaufmann and Curtis C. Harris, M.D.

Cooperating Units: C.W. Dingman, M.D., Nucleic Acids Section, Chemistry Branch, NCI; B. I. Gerwin, Ph.D., Viral Leukemia and Lymphoma Branch, NCI: J.W. Grisham, M.D., University of North Carolina School of Medicine, Chapel Hill, N.C.

Man Years:

Total	:	1.6
Professional	:	0.6
Other	:	1.0

Project Description

Objectives:

The aim of this project is to determine features of eukaryotic DNA synthesis and the control of this process. Knowledge of these processes would be valuable since abnormality of growth control appears to be a general property of malignant tissues. An understanding of the acute and subacute effects of carcinogens on DNA synthesis and its control processes in normal cells is the ultimate objective of these studies.

Methods Employed:

Partial hepatic resection in rats has been employed to provide a partially synchronized cellular population of normal cells in vivo without use of chemical synchronization methods. Brief or prolonged intervals of labeling in vivo with thymidine-^{3}H (TdR-^{3}H) have been used to preferentially label nascent or non-nascent DNA. Nuclei have been isolated from liver cells by gentle methods and have either been used in in vitro DNA synthesis assays or disrupted and futher fractionated. Methods employed for fractionation of nuclear constituents and purified DNA, included isopyknic gradient centri-

fugation, hydroxyapatite chromatography, and agarose-acrylamide composite gel electrophoresis. Electron microscopy has been used to study DNA and nuclear constituents and sophisticated high resolution autoradiography techniques have been used to determine distribution of radioactivity.

Major Findings:

DNA has been synthesized in vitro in isolated rat liver nuclei. DNA repair was present in nuclei of both S-phase and non-replicating cells and was an ATP-independent process in this system. In contrast, replicative DNA synthesis was dependent on ATP and demonstrable only in nuclei of cell populations containing S-phase cells. These two processes also had different properties in terms of the effects of substrates, inhibitors and co-factors. In a separate series of experiments, nuclei were isolated from liver following in vivo labeling with TdR-^{3}H, then sonicated, and fractionated by isopyknic gradient centrifugation. Nuclear constituents were separated into three principal fractions (LB, MB, and HB). Brief in vivo labeling with TdR-^{3}H resulted in higher specific activity in LB and MB DNA than in HB. After longer labeling periods, most radioactivity and the highest specific activity were in the HB. The labeled DNA in the LB and MB was partially single stranded and contained portions which are retarded on acrylamide gel electrophoresis like replication forks. DNA polymerase activity was demonstrable in gradient fractions; the greatest quantities were in the LB and MB. Only 3S polymerase activity was found in nuclei and nuclear fractions, and this polymerase activity varied with the stage of the cell cycle. DNA polymerase activity primed with endogenous DNA and dependent on ATP was predominently in the MB with a smaller quantity in the LB. When DNA was replicated in vitro in whole isolated nuclei and the products analyzed by the density gradient method, ATP-dependent DNA replication was once again found only in the MB and LB.

Significance to Biomedical Research and the Program of the Institute:

These studies contribute to the basic understanding of the processes of DNA replication and the repair of DNA damage. The control of DNA replication and cell proliferation, and the repair of carcinogen induced damage to DNA, are currently significant subjects for investigations directed toward elucidation of the basic mechanism of carcinogenesis. These studies have been conducted in tissues and systems in which they are most readily and easily investigated.

Proposed Course of Project:

Current studies will be brought to their conclusions and the results will be reported.

Publications

Genta, V.M., Kaufman, D.G., and Kaufmann, W.K.: A method for fractionating rat liver cell nuclei on equilibrium density gradients of Cs_2SO_4. Anal. Biochem., in press.

Project No. Z01-CP-04553-03-LC
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Effects of Vitamin A and Analogs on Cell Cultures of Epidermis

Previous Serial Number: Same

Principal Investigators: Michael B. Sporn, M.D., and Nancy Dunlop, M.S.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	0.5
Professional	:	0.1
Other	:	0.4

Project Description

Objectives:

The aim of the project is to use epidermal cell cultures as a primary screen for evaluation of biological activity of new synthetic vitamin A analogs, as well as for further elucidation of the mechanism of action of vitamin A.

Methods Employed:

The new methods developed by Yuspa and collaborators for culture of pure populations of mouse epidermal cells are being used. These have been modified in the Lung Cancer Branch so that it is now possible to work in serum-free media.

Major Findings:

A large number of vitamin A analogs have been screened in this test system, and correlations have been made with their activity in the tracheal organ culture system. In general, there is a good correlation of activity, although there are significant exceptions, such as for retinoic acid ethylamide and retinyl-N-acetyl amine, both of which are active in the tracheal system and are inactive in the skin system. These findings suggest

that vitamin A analogs must be metabolically activated before they are physiologically functional. The relatively non-toxic compound, retinyl methyl ether, has been found to be particularly active in the skin cell culture system. This finding has suggested the synthesis of still other retinyl ethers, which will in turn be screened for *in vitro* and *in vivo* activity.

Significance to Biomedical Research and the Program of the Institute:

Since the eventual use of vitamin A analogs for cancer prevention in man is currently under consideration, it is of utmost importance to have the most efficient primary screen for assessment of activity. This system will provide such a screen.

Proposed Course of Project:

To continue as outlined above.

Publications

Sporn, M.B., Clamon, G.H., Dunlop, N.M., Newton, D.L., Smith, J.M., and Saffiotti, U.: Activity of vitamin A analogues in cell cultures of mouse epidermis and organ cultures of hamster trachea. Nature 253: 47-50, 1975.

Project No. Z01-CP-04566-02-LC
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Susceptibility States and Human Lung Cancer

Previous Serial Number: None

Principal Investigator: Curtis C. Harris, M.D.

Other Investigators: Martin Cohen, M.D., Aron Primack, M.D., Richard Talamo, M.D., and Geno Saccomanno, M.D., Ph.D.

Cooperating Units: NCI-VA Oncology Branch, DCT, NCI; Department of Pediatrics, Johns Hopkins School of Medicine; St. Mary's Hospital, Grand Junction, Colorado.

Man Years:

Total	:	0.1
Professional	:	0.1
Other	:	0.0

Project Description

Objectives:

The purpose of the present study was to determine if individuals with lung cancer have an alpha$_1$-antritrypsin (AAT) deficiency, which could be a predisposing factor to the oncogenic effects of either cigarette smoke or other environmental carcinogens.

Methods Employed:

Clinical Material - Seventy-three patients with non-resectable and histologically proven lung cancer (70 male, 3 female) were studied at the Washington Veterans Administration Hospital. Their mean age was 56 years (range 40-83) and 72% of the patients were Caucasian. They had smoked cigarettes for an average of 30 pack years, i.e., average number of packages of cigarettes smoked per day multiplied by the number of years of smoking was 30. Patients, without lung cancer, hospitalized at the same institution served as controls. The control patients were matched according to age, sex, and smoking history to the 26 patients with squamous cell carcinoma of the lung. A population of patients without lung cancer is also being studied. The sputum of these individuals has been examined and any abnormalities classified according to the scheme described by Saccomanno.

AAT Levels - The serum was stored at -20° until radio-immunodiffusion assay by the Mancini method. The accuracy of this assay is ± 10%. All serum specimens were coded and assayed in a blind fashion.

AAT Phenotypes - The phenotypes of AAT were determined on acid starch gel electrophoresis by a modified method of Fagerhol.

Major Findings:

Serum levels of AAT were significantly elevated in the four major cell types of lung cancer, when compared to control levels. While the highest level was found in patients with large cell anaplastic carcinoma, the levels were not significantly different between the four histological types of cancer. The mean AAT level in hospitalized patients without lung cancer, who served as controls, was the same as that found in the general population. Patients with primary cancer at other sites also had elevated levels. Thus, malignancy is one of the several exogenous and endogenous factors that increase serum $alpha_1$-antitrypsin.

The phenotypes of AAT are under genetic control. They are not affected by exogeneous factors and may be a more informative guide to susceptibility. The determination of AAT phenotypes in lung cancer patients as well as patients without lung cancer (but with abnormal sputum cytology) is in progress.

Significance to Biomedical Research and the Program of the Institute:

The identification of individuals in the population who are highly susceptible to the oncogenic effects of cigarette smoke may allow active intervention and prevention of lung cancer.

Proposed Course of the Project:

Continuation of studies outlined above.

Publication

Harris, C.C., Primack, A., and Cohen, M.: $Alpha_1$-antitrypsin levels in the serum of patients with lung cancer. Cancer 34: 280-281, 1974.

Project No. Z01-CP-04567-02-LC
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Carcinogenesis Studies in Human Tissues

Previous Serial Number: Same

Principal Investigators: Curtis Harris, M.D., and Benjamin Trump, M.D.

Other Investigators: Carolien van Haaften, Ph.D., Arthur Frank, M.D., Elizabeth McDowell, D.V.M., Ph.D., Lucy Barrett, Ph.D., Mariam Valeria, D.V.M., Ph.D., Elliott Fineman, Frank Jackson, and Maria Yamaguchi.

Cooperating Units: Ultrastructure Unit, Experimental Pathology Branch, CG; Department of Pathology, University of Maryland, Baltimore, Maryland; Litton-Bionetics, Kensington, Maryland.

Man Years:

Total	4.5
Professional	3.0
Other	1.5

Project Description

Objectives:

To develop experimental systems to study carcinogenesis in human target tissues under controlled conditions. Such a system involves 3 major facets: 1) obtaining of viable human tissues; 2) *in vitro* maintenance of epithelial cells in these tissues in cell and organ cultures; and 3) xenotransplantation of human tissues into immunodeficient animals.

Methods Employed:

Human tissues are obtained at either surgery or "immediate" autopsy. The tissue is both maintained in organ culture and xenotransplanted into athymic nude mice. These tissues are exposed to chemical carcinogens *in vitro* and/or as a xenograft. The morphological lesions caused by carcinogens are examined by light and electron microscopy. Binding of carcinogens to macromolecules in epithelial cells is measured by biochemical and autoradiographic methods, which were previously developed, in part, in studies of animal models.

Major Findings:

Significant progress has been made in each of the three major technical facets of this program: 1) Obtaining and Storage. Grossly normal-appearing bronchi from patients with or without lung cancer, who have not previously received cancer therapy, were obtained at either surgery or "immediate" autopsy. The bronchial specimens can be successfully stored at 4°C in L-15 medium for at least 6 days prior to culture. 2) Organ Culture. Human bronchi can be cultured in vitro for at least 4 weeks in CMRL 1066 medium containing 1 µg/ml crystalline insulin, 0.1 µg/ml hydrocortisone hemisuccinate, 0.1 µg/ml beta-retinyl acetate, 100 u/ml penicillin G, 2 mM L-glutamine and 100 µg/ml streptomycin, and for at least 15 weeks in medium supplemented with 5% heat-inactivated fetal calf serum. For the first 6 weeks, the bronchial epithelial cells retain essentially the normal ultrastructure of those cells studied prior to culture. They actively incorporate precursors into DNA, RNA and protein. Ultrastructural cytochemical studies have shown that the surface of the cultured epithelial cells has properties similar to uncultured epithelial cells. After culture for 7 or more weeks, the epithelium becomes progressively more hyperplastic and contains few ciliated cells. 3) Xeno-transplantation. Human bronchial specimens, i.e., 1 x 1 cm pieces and sub-segmental bronchi, can be maintained as xenografts in athymic nude mice for at least 4 months without evidence of immune rejection. Epithelium, bronchial glands and mesenchyme are well maintained. After a period of ischemia followed by epithelial regeneration, the epithelium attains of fully differentiated state with mucous and ciliated cells lining the lumen of the xenograft. Unlike those in the athymic nude mice, xenografts in neonatally-thymectized hamsters are rejected within 2 weeks. If these hamsters are further immunosuppresed with anti-lymphocyte serum, xenografts can be maintained for at least 1 month. However, infiltration of lymphocytes into the xenograft is present.

Another important aspect of these investigations has been to study the metabolism of known and suspected environmental carcinogens in cultured human bronchial mucosa. Current studies concern the binding of ^{3}H-PNH (7,12-dimethylbenz[a]anthracene [DMBA]; 3-methylcholanthrene; benzo(a)pyrene (BP); and dibenz(a,h)anthracene) to cellular macromolecules. After 7 days, ^{3}H-PNH were dissolved in dimethylsulfoxide and added to the cultures for periods of 4, 12, or 24 hr. Binding of ^{3}H-PNH to DNA was measured biochemically following isolation of DNA on CsCl gradients and binding to total macromolecules was determined by quantitative autoradiography (ARG). ARG allows the cellular localization of bound PNH in a tissue containing a mixed population of cells in varying states of differentiation. For example, more bound ^{3}H-PNH were localized by ARG in epithelial cells than in mesenchymal cells. ^{3}H-DMBA binds to DNA to a greater extent than the other ^{3}H-PNH studied. Binding of ^{3}H-BP to DNA increased with either increasing exposure time or increasing dose of BP (0.15, 1.5 and 15 µM). Binding of ^{3}H-BP for 3 hr was 75% less at 0°C than at 37°C.

A variety of synthetic and naturally-occurring compounds are known to alter the metabolism of PNH and in some cases inhibit their tumorigenicity in experimental animals. These compounds may provide one means of intervention to prevent human cancer. Selected chemicals are being studied in our in vitro

system employing the human bronchial mucosa. Either butylated hydroxytoluene (2 or 10 μM) or 7,8-benzoflavone (20 μM) inhibited ^{3}H-BP (1.5 μM) binding to DNA by 60%. Nicotine (0.1, 1 and 10 μM) did not significantly alter the level of bound BP. Many environmental chemicals, including PNH, require enzymatic activation to reach full carcinogenic potential. Thus the ability to form carcinogenic metabolites could be an important susceptibility factor in man. Preliminary data suggest that bronchial mucosal samples from patients with squamous cell carcinoma bind ^{3}H-PNH to their DNA 2-4 fold more than bronchial mucosa from patients with either adenocarcinoma or no lung cancer.

Significance to Biomedical Research and the Program of the Institute:

The extrapolation of carcinogenesis data from studies utilizing experimental animals to man presents several complex problems. One approach to provide a link between these experimental data and human cancer is the development of an experimental system to study carcinogenesis in important human target tissues. Such a system could aid in the identification of individuals who are highly susceptible to environmental carcinogens, and methods of intervention could be tested in this experimental system prior to clinical trials in man.

Current results also indicate that carcinogen metabolism can be studied directly in human target cells e.g., bronchial epithelial cells.

Proposed Course of Project:

Attempts are continually being made to improve each interlocking facet of this program. Conditions for organ culture of human bronchi are being modified to prevent the hyperplasia associated with prolonged culture. Studies have been initiated to develop cell cultures derived from human bronchial epithelial cells.

A prospective epidemiological-laboratory study has been initiated in an attempt to confirm and delineate the observations that the non-tumorous bronchial epithelium from patients with squamous cell carcinoma of the lung bind carcinogenic polynuclear hydrocarbons to their DNA at a higher level than bronchial epithelium from patients either without lung cancer or with adenocarcinoma of the lung. Finally, the methodologies developed for maintaining and studying human bronchi under controlled experimental conditions will be used to develop similar experimental systems for other important human target tissues, e.g., pancreatic duct.

Honors and Awards

Invited Lecture, Conference, The Delayed Consequences of Cancer Therapy: Proven and Potential, Sponsored by Cancer Clinical Investigation Review Committee, Orlando, January 9, 1975.

Coordinator and Lecturer, Third Annual Carcinogenesis Collaborative Conference, Advances in Utilizing Human Cells and Tissues in Carcinogenesis, Sponsored by DCCP, NCI, Orlando, February 3, 1975.

Invited faculty. Special Course, Electron Microscopy in Diagnostic Pathology, Sponsored by International Academy of Pathology, New Orleans, March 8, 1975.

Member, Lung Cancer Segment, DCCP, NCI

Member, Working Party for Therapy of Lung Cancer, DCT, NCI.

Member, Organizing Committee, International Conference on Late Effects of Cancer Therapy, January 9-11, 1975, sponsored by Cancer Clinical Investigations Review Committee, NCI.

Member, Ad Hoc Scientific Advisory Group on In Vitro Carcinogenesis, DCCP, NCI.

Publications

Harris, C., Genta, V., Frank, A., Kaufman, D., Barrett, L., McDowell, E., and Trump, B.: Carcinogenic polynuclear hydrocarbons bind to DNA in cultured human bronchi. Nature 252: 68-69, 1974.

Trump, F.B., McDowell, E.M., Barrett, L.A., Frank, A., and Harris, C.: Studies of ultrastructure, cytochemistry and organ culture of human bronchial epithelium. In Karbe, E. and Park, J. (Eds.): Experimental Lung Cancer. Heidelberg, Springer-Verlag, 1974, pp. 548-564.

Kaufman, D., Genta, V., and Harris, C.: Studies on Ccarcinogen binding in vitro in isolated hamster tracheas. In Karbe, E. and Park, J. (Eds.): Experimental Lung Cancer. Heidelberg, Springer-Verlag, 1974, pp. 564-574.

Genta, V., Kaufman, D., Harris, C., Jackson, F., Smith, J., Sporn, M., and Saffiotti, U.: Vitamin A deficiency enchances the binding of benzo(a)pyrene to tracheal epithelial DNA. Nature 247: 48-49, 1974.

Harris, C.: Immunosuppressive anticancer drugs in man: Their oncogenic potential. Radiology 114: 163-167, 1975.

Harris, C.: The carcinogenicity of anticancer drugs: A hazard in man. Cancer, in press.

Meadows, A., D'Angio, G., Evans, A., Harris, C., Miller, R., and Mike, U.: Oncogenesis and other late effects of cancer treatment in children. Radiology 114: 175-181, 1975.

Yuspa, S., and Harris, C.: Altered differentiation of mouse epidermal cells treated with retinyl acetate In Vitro. Exp. Cell Res. 86: 95-105, 1974.

Wenk, M., Reddy, J., and Harris, C.: Pancreatic regeneration in the guinea pig caused by ethoinine. J. Natl. Cancer Inst. 52: 533-538, 1974.

Boren, H., Pauley, J., Wright, E., Kaufman, D., Smith, J., and Harris, C.: Cell populations in the hamster tracheal epithelium in relation to vitamin A status. Int. J. Vitam. Nutr. Res. 44: 382-390, 1974.

Project No. Z01-CP-04570-02-LC

1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Intranuclear Distribution of DNA Repair Synthesis and Ultrastructural Lesions in Human Diploid Cells Induced by Ultimate Chemical Carcinogens or Ultraviolet Light

Previous Serial Number: Same

Principal Investigator: Curtis Harris, M.D.

Other Investigators: Michael Lieberman, M.D., Robert Conner, Ph.D., Frank Jackson and Maria Yamaguchi

Cooperating Units: Ultrastructure Unit and Digestive System Carcinogenesis Section, Experimental Pathology Branch, CG, Biometry Branch, Demography

Man Years:

Total	0.6
Professional	0.2
Other	0.4

Project Description

Objectives:

Chemical carcinogens and ultraviolet light damage nuclear DNA, which then may be repaired by enzymatic synthesis. The intranuclear distribution of DNA repair synthesis is unknown. The aim of this project is to determine this distribution as well as to examine the concurrent changes in nuclear ultrastructure.

Methods Employed:

Treatment of Cells. WI-38 human diploid fibroblasts were grown to confluence in diploid growth medium containing neomycin 73.5 mg/liter and 10% fetal calf serum. These contact-inhibited cells were exposed to either: 1) NAAAF (1 or 10 μm) or 7-BrMeBA (100 μm) in redistilled dimethyl sulfoxide (final concentration 0.5%), NMU (0.2, 1 or 2 mM; 2) UVL (200 erg/sqmm) administered at a rate of 14 erg/mm^2/sec from a low pressure mercury lamp; 3) control cultures received dimethyl sulfoxide along. Fifteen minutes after treatment, ^{3}H-methyl TdR (10 μCi/ml; sp. activity 50 Ci/mmol; New England Nuclear, Boston, Massachusetts) was added. Hydroxyurea was not added to the culture medium.

Quantitation of Autoradiograms. In autoradiograms of 1 μ sections of cell pellets, the randomness of the intranuclear distribution of the autradiographic grains was investigated by two quantitative statistical approaches. The first considered randomness versus a general non-randomness while the second looked specifically at the difference in grain density between the inner and outer portion of the nucleus.

Major Findings:

Confluent, non-S-phase human diploid fibroblasts (WI-38) showed autoradiographic evidence of DNA repair synthesis following exposure to either N-methyl-N-nitrosourea, 7-bromomethylbenz(a)anthracene, N-acetoxy-2-acetylaminofluorene, or ultraviolet light (predominantly 254 nm) and subsequent labeling with thymidine-^{3}H. The intranuclear distribution of DNA repair synthesis (unscheduled DNA synthesis) in individual cells was nonrandom, i.e., did not fit a random model (Poisson distribution). We compared the autoradiographic grain density near the nuclear envelope ("outer shell") to the density in the central nuclear area ("inner core"). When the grain densitites were corrected for the greater packing of DNA in the outer shell, there was an approximately 2-fold increase in grain densities of the inner core when compared to the outer shell. By electron microscopy the DNA in the inner core is primarily euchromatic, while that in the outer shell is primarily heterochromatic. No alterations in either the ultrastructure of heterochromatin or its peripheral distribution were observed in cells undergoing DNA repair synthesis at doses of carcinogens examined. The major ultrastructural change was segregation of nucleolar granules and fibrils in cells exposed to either N-acetoxy-2-acetylaminofluorene, ultraviolet light, or 7-bromomethylbenz(a)-anthrachene.

Significance to Biomedical Research and the Program of the Institute:

Chemical carcinogens and UVL damage DNA, causing induction of DNA repair synthesis in many types of mammalian cells. The damage by chemical carcinogens is thought to be primarily due to their alkylating DNA. Inaccurate or incomplete DNA repair synthesis of damaged DNA could lead to neoplastic transformation. This project may aid in the understanding of DNA repair synthesis and neoplastic transformation.

Proposed Course of the Project:

Project has been completed.

Publication

Harris, C., Connor, R., Jackson, F., and Lieberman, M.: The intranuclear distribution of DNA repair synthesis induced by chemical carcinogens or ultraviolet light in human diploid fibroblasts. Cancer Res. 34: 3461-3469, 1974.

Project No. Z01-CP-04571-02-LC
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on the Role of Asbestos in Causation of Lung Cancer

Previous Serial Number: Same

Principal Investigators: Arthur L. Frank, M.D., Michael B. Sporn, M.D., and Joseph M. Smith

Other Investigators: David G. Kaufman, M.D., Ph.D.

Cooperating Units: Lewis Lipkin, M.D., and Morton Schultz, Image Processing Unit, Division of Cancer Treatment, NCI.

Man Years:

Total	1.3
Professional	0.9
Other	0.4

Project Description

Objectives:

To study biological effects of asbestos. The objectives of this research are to elucidate the effects of asbestos on tissues, especially epithelial tissue, and to elucidate what mechanisms are operative in the causation, by asbestos, of lung cancer, mesothelioma, colon, and rectal cancer.

Methods Employed:

Whole animals: After initial studies to determine what dosages could be given to hamsters by tracheal instillations groups of animals were dosed and have been followed for development of pathologic changes. Though after 1 year no tumors have been noted in any of the animals so far examined, changes such as hyperplasia and fibrosis have been noted. Plans are being made for a co-carcinogenic study of asbestos and benzpyrene instillations together in a large group of animals.

Organ Culture - Hamster: Using techniques developed in the Lung Cancer Branch hamster tracheas are being successfully maintained in organ culture. In addition to the practice of exposing the tracheas to various forms of asbestos, with resultant quantitatively measured changes, other materials are being

investigated including talc, diatomaceous earth activated charcoal, and altered forms of asbestos which have lost their ability to be active in a hemolytic test system. Also being investigated are dose response curves of asbestos, and of retinyl acetate.

Organ Culture - Human: Cultured human bronchus is being exposed to asbestos to determine if there is any effect of this exposure on binding of tritiated polycyclic hydrocarbons to DNA.

EM and Scanning EM - Studies have been under taken to examine cultured tissues exposed to asbestos for morphologic changes.

Cell Counting - Using the NCI Grain Counter I developed by Dr. Lewis Lipkin and Morton Schultz quantitative measurements have been made on cultured hamster epithelial cells. Plastic embedded sections are being used for counting on this apparatus.

Major Findings:

It has been shown that amosite asbestos causes a hyperplastic response on organ cultured hamster tracheal epithelium. Within 24 hours there is an increase in the number of cells present per unit length of about 15%. This finding appears to be reproducible and consistent. Yet to be examined are chrysotile asbestos exposed specimens, and other non-asbestos materials. Radioautographic methods are being worked on to confirm these findings.

Significance to Biomedical Research and the Program of the Institute:

A system is now established with consistent and reproducible changes caused by asbestos, and investigations of modifying influences can now be investigated.

Proposed Course of Project:

To continue description of the basic system, investigate other materials, and evaluate potential anti-carcinogenic agents.

Project No. Z01-CP-04572-01-LC
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Co-carcinogenic Effects of Azoxymethane and Asbestos

Previous Serial Number: None

Principal Investigator: Arthur L. Frank, M.D.

Other Investigators: Jerrold M. Ward, D.V.M., Ph.D., Martin Wenk, Ph.D.

Cooperating Units: Microbiological Associates

Man Years:

Total	:	0.1
Professional	:	0.1
Other	:	0.0

Project Description

Objectives:

Asbestos appears to have considerable co-carcinogenic activity in relation to cancer of the lung, and colon and rectal cancer. This study was designed to see what effect feeding asbestos would have on animals treated with a known colon carcinogen.

Methods Employed:

Groups of rats were given sub-cutaneous injections of azoxymethane, a known colon carcinogen. Some of these animals were also given by oral intubation either amosite or chrysotite asbestos. Parameters to be measured include change in incidence, site, or time of onset in expected tumors.

Major Findings:

This study is in progress and all that is known at present is that all groups tolerated the initial 10 week dosing procedures and are now being observed for tumor production.

Significance to Biomedical Research and the Program of the Institute:

This work may demonstrate the co-carcinogenic effects of asbestos with a known carcinogen.

Proposed Course of Project:

This project will be continued at Mount Sinai in New York.

Project No. Z01-CP-04573-01-LC
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Effect of Vitamin A on the Control of Bladder Carcinogenesis

Previous Serial Number: None

Principal Investigator: David H. Reese, Ph.D.

Other Investigators: Joseph M. Smith, Dianne L. Newton

Cooperating Units: None

Man Years:

Total	:	1.0
Professional	:	0.8
Other	:	0.2

Project Description

Objectives:

The purpose of this study is to develop another system (urinary bladder) which can be used for comparison with the trachea on the problem of reversal of preneoplasia with vitamin A analogs (retinoids). The scope of this study encompases: (1) Development of an organ culture system for the rat urinary bladder, (2) Production of preneoplastic lesions in rat urinary bladders and (3) Assay of the effect of retinoids on preneoplastic lesions in rat urinary bladder in organ culture.

Methods Employed:

Urinary bladders from Fischer and Osborne-Mendel rats have been cultured under a variety of organ culture conditions to determine the most optimum conditions for culture. The effectiveness of the carcinogen N-methyl-N-nitrosourea (NMU) in producing preneoplastic lesions in Osborne-Mendel rats has been tested.

Major Findings:

The culture medium CMRL-1066, which has been used successfully for trachea culture has not been adequate for bladder culture. Two other media, Eagle's minimum essential medium and Ham's F-12, have both proved satisfactory however. The latter medium, in addition to maintaining the tissue in culture,

causes an approximately 200-fold increase in basal cell labelling with thymidine after 2 days in culture. NMU, after only 2 treatments, produces extensive hyperplasia and squamous metaplasia. NMU-treated bladders have not been cultured successfully in excess of 5 days, probably because of a severe inflammatory response. The epithelium is maintained past this time but the muscle is not.

Significance to Biomedical Research and the Program of the Institute:

It is hoped that this project will provide another epithelial system on which the control of preneoplasia by retinoids can be tested.

Proposed Course of Project:

Other carcinogens will be tested for their ability to produce preneoplastic lesions in the bladder which can be maintained in culture for extended periods of time. Procedures will then be as outlined under objectives.

Project No. Z01-CP-04697-07-LC
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Experimental Respiratory Carcinogenesis

Previous Serial Number: Same

Principal Investigator: David G. Kaufman, M.D., Ph.D.

Other Investigators: Umberto Saffiotti, M.D., Curtis C. Harris, M.D.

Cooperating Units: Russell Madison, D.V.M. and Steven Springer, Microbiological Associates, Inc.; and Bruce Zwilling, Ph.D. Ohio State University

Man Years:

Total	: 0.3
Professional	: 0.3
Other	: 0.0

Project Description

Objectives:

The aim of this project is to elucidate causative factors and pathogenetic mechanisms of respiratory tract cancer and to define biological models for the experimental study of bronchogenic carcinoma.

Methods Employed:

Carcinogens are administered to Syrian hamsters by intratracheal instillations: (a) Benzo[a]pyrene (BP) as a particulate suspension attached to particles of ferric oxide (Fe_2O_3); and (b) Nitrosomethylurea (NMU) or (c) β-Propiolactone (BPL) dissolved in buffered saline. Experimental groups are designed to consider following points: (1) the carcinogenicity of various single or multiple dose regimens of NMU in the respiratory tract, (2) the carcinogenicity of BPL in the respiratory tract, (3) the synergistic or co-carcinogenic effects of administration of NMU (or dilute HCl) in addition to BP plus Fe_2O_3, and (4) the effects of treatments with either vitamin A or Ribi Vaccine on groups of hamsters to which a respiratory-tract carcinogenesis regimen had been administered. Results are to be derived from analysis of the gross and microscopic lesions observed at death in experimental animals.

Project No. Z01-CP-04697-07-LC

Major Findings:

NMU alone, administered intratracheally, caused a low incidence of respiratory tract tumors for all doses and numbers of treatments tested. Respiratory tract tumors were primarily non-malignant papillary tumors of the trachea and major bronchi. Tumors of other organs were observed, with colon the most common site. Regimens of BPL alone, administered intratracheally have not produced respiratory tract tumors in experiments still in progress. Multiple intratracheal instillations of BP plus Fe_2O_3, followed by multiple doses of NMU, results in a synergistic increase in tumor response when compared with the sum of tumors with BP plus Fe_2O_3 alone plus NMU alone. Tumors resembled those produced by BP plus Fe_2O_3 alone. Examination of the histogenesis of squamous cell carcinoma of the laryngo-tracheobronchial epithelium in these protocols indicates that a spectrum of changes, perhaps stages in the transition to malignant lesions, can be identified: a) hyperplasia, b) regular squamous metaplasia, c) dysplasia with focal basal cell hyperplasia, d) nodular overgrowth of basal cells, e) pseudsepitheliomatous hyperplasia, f) in situ carcinoma, and g) invasive squamous carcinoma. Analysis of the results of an experiment in which intratracheal treatments with BP plus Fe_2O_3 were followed by subcutaneous diethylnitrosamine (DEN) showed synergistic induction of malignant tumors of the tracheobronchial tract as compared with treatment with BP plus Fe_2O_3 alone. Comparable tumors of the tracheobronchial tract are not seen in hamsters treated with DEN alone. Hamsters treated intratracheally with BP plus Fe_2O_3 also received retinol acetate both during and after treatment with the carcinogen. As compared with a comparable group treated with BP plus Fe_2O_3 but no retinol acetate, there was a marked reduction in the tumor incidence (or an increase in latency) in the group treated with retinol acetate. It has been demonstrated previously that hamsters injected intravenously with Ribi vaccine (BCG cell-wall preparation) become immunized. Hamsters treated intratracheally with BP plus Fe_2O_3 received intravenous injections of either: a) saline, b) (single or multiple doses) Ribi Vaccine or c) vehicle for suspending Ribi vaccine, after the completion of carcinogen treatment. Preliminary results to the present time show a decreased incidence of respiratory tract tumors in the groups treated with Ribi Vaccine as compared to saline or vehicle controls.

Significance to Biomedical Research and the Program of the Institute:

These studies are part of the efforts aimed at defining factors leading to the development of bronchogenic carcinoma in animal systems under conditions that resemble those of human lung cancer development. Studies in progress have the following as objectives: (1) development of protocols with which to study the tumorigenic interactions between two carcinogens, (2) development of a carcinogenic protocol which would induce respiratory tract tumors in high incidence and with short latency, and (3) determine whether either vitamin A or Ribi Vaccine can modify (reduce) the lung tumor incidence in hamsters treated with current respiratory carcinogenesis regimens.

Proposed Course of Project:

Long term carcinogenesis studies currently in progress will be brought to their conclusions and results will be reported.

Project No. Z01-CP-04697-07-LC
Publications

Montesano, R., Saffiotti, U., Ferrero, A., and Kaufman, D.G.: Synergistic effects of benzo[a]pyrene and diethylnitrosamine on respiratory carcinogenesis in hamsters. J. Natl. Cancer Inst. 53: 1395-1397, 1974.

Saffiotti, U., and Kaufman, D.G.: Carcinogenesis of laryngeal carcinoma. Laryngoscope 85; 454-467, 1975.

Kaufman, D.G., and Madison, R.M.: Synergistic effects of benzo[a]pyrene and N-methyl-N-nitrosourea on respiratory carcinogenesis in Syrian golden hamsters. In Karbe, E. and Park, (Eds.): Experimental Lung Cancer. Heidelberg, Springer-Verlag, 1974, pp. 575-582.

Project No. Z01-CP-04792-05-LC
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Morphogenesis of Squamous Cell Carcinoma of the Respiratory Tract in Man and Animal Models

Previous Serial Number: Same

Principal Investigators: Curtis C. Harris, M.D., Benjamin Trump, M.D., and Umberto Saffiotti, M.D.

Other Investigators: David G. Kaufman, M.D., Ph.D., Curtis Port, D.V.M., Ph.D., Elizabeth McDowell, D.V.M., Ph.D., Frank Jackson, Maria Yamaguchi, and Joseph M. Smith

Cooperating Units: Ultrastructure Unit, Experimental Pathology Branch, CG; Department of Pathology, University of Maryland, Baltimore, Maryland; IIT Research Institute, Chigago, Illinois

Man Years:

Total	:	0.6
Professional	:	0.2
Other	:	0.4

Project Description

Objectives:

Previous investigations of the histogenesis of squamous cell carcinoma in man and animal models have shown that respiratory epithelium becomes hyperplastic, squamous metaplastic and finally neoplastic. These sequential changes in morphology may be better understood and defined by ultrastructural analysis.

Methods Employed:

In patients, with suspected lung cancer, grossly normal and abnormal bronchial areas are biopsied using a fiber-optic bronchoscope. The bronchial specimens taken at surgery from patients with lung cancer is also studied. The bronchial tissue is examined by high-resolution light and electron microscopic study.

Major Findings:

Respiratory epithelium was examined by high-resolution light and electron microscopy in patients with suspected lung cancer and in animal models of bronchogenic carcinoma. In human bronchial epithelium, atypical squamous metaplastic areas showed widened intercellular spaces, pleomorphic nuclei and abnormal basal laminae. Similar results were found in serial-sacrifice studies in animal models. In addition, atypical cilia were seen in cells adjacent to squamous metaplastic cells in the respiratory epithelium of man and the animal models. Intracellular, compound and intercellular cilia were found. The ultrastructure of squamous cell carcinoma, adenocarcinoma and anaplastic carcinoma of the lung in the animal models was similar to that of neoplastic tumors of the same histological types found in man. Current data are consistent with the hypothesis that basal cell hyperplasia precedes squamous metaplasia, which can then be followed by squamous cell carcinoma. Squamous metaplasia caused by noncarcinogenic agents and states is being compared to that caused by chemical carcinogens in animal models. Significant differences between squamous metaplasia caused by carcinogens and that found during vitamin A deficiency have been observed by both scanning and transmission electron microscopy.

Significance to Biomedical Research and the Program of the Institute:

The incidence of bronchogenic squamous cell carcinoma in man has shown a progressive increase during the past 5 decades. The pathogenesis of bronchogenic carcinoma is being studied in animal models and in man by several experimental approaches by members of the Lung Cancer Branch. This multi-faceted approach allows meaningful correlation of morphological and biochemical changes occurring during carcinogenesis. Intramural investigations are closely coordinated with the activities of the Lung Cancer Segment.

Proposed Course of the Project:

Continuation of comparative studies between the morphological lesions found in man and those observed in animal models. The current classification of human lung cancer is being re-evaluated by improved morphological and cytochemical techniques.

Publications

Harris, C.: Cause and prevention of lung cancer. Semin. Oncol. 1: 163-167, 1974.

Harris, C., Kaufman, D., Jackson, F., Smith, J., Dedick, P., and Saffiotti, U.: Atypical cilia in the tracheobronchial epithelium of the hamster during respiratory carcinogenesis. J. Path. 114: 17-19, 1974.

Port, C.D., Baxter, D.W., and Harris, C.C.: Surface morphology of tracheal epithelium in vitamin A deficiency and reversal. In Karbe, E. and Park, J. (Eds.): Experimental Lung Cancer. Heidelberg, Springer-Verlag, 1974, pp. 575-582.

Project No. Z01-CP-04793-05-LC
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: *In Vitro* Metabolic Studies in Isolated Hamster Respiratory Tract Tissue

Previous Serial Number: Same

Principal Investigator: David G. Kaufman, M.D., Ph.D.

Other Investigators: Curtis C. Harris, M.D.

Cooperating Units: Carl Johnson, Ph.D., Microbiological Associates, Bethesda, Maryland

Man Years:

Total	:	0.1
Professional	:	0.1
Other	:	0.0

Project Description

Objectives:

The general aim of this project is to examine metabolic processes in a respiratory epithelium. The specific current objective of these studies is to determine whether a short-term *in vitro* assay can be established which will have a high rate of correlation with the results of long-term carcinogenesis studies. It is hoped that such an assay might indicate situations affecting the susceptibility of the hamster respiratory tract to tumor induction and provide experiments with more rapid turnover than life-time studies.

Methods Employed:

Techniques have been developed for maintaining isolated hamster tracheas *in vitro* as organ cultures for at least 4 hours. Short periods of maintenance were specifically chosen so that conditions *in vitro* reflect the *in vivo* state as well as possible. Previous studies have shown that radioisotopically labeled nucleic acid precursors added to the incubation medium could be recovered in well preserved high molecular weight RNA obtained from tracheas maintained *in vitro*.

Major Findings:

The current series of studies have shown that tracheas incubated in vitro in medium with benzo[a]pyrene-^{3}H (BP-^{3}H) had BP-^{3}H bound to the purified DNA extracted from the epithelial cells and banded in cesium chloride gradients. Prior intratracheal treatment of hamsters with BP plus Fe_2O_3 in vivo resulted in increased in vitro binding of BP-^{3}H to tracheal DNA. This enhanced binding was inhibited by incubation in the presence of 7,8-benzoflavone; these results suggest that binding may be composed of two components, one inhibitable by 0° temperature but not by 7,8-benzoflavone, whereas the induceable component appears completely inhibitable by 7,8-benzoflavone. Preliminary evidence suggests that there is an inverse relationship between hamster age (in young hamsters) and induceable binding levels. There was also increased binding observed in certain inbred hamster strains. Tracheas from vitamin A deficient hamsters had greater quantities of bound BP-^{3}H than their pair-fed vitamin A normal controls. Addition of retinol acetate to the medium during in vitro incubation partially inhibits this increased binding in tracheas from eficient hamsters. Furthermore, this inhibition of binding is not due to a general toxic effect of the retinol acetate. The question of whether these results are relevant to, or predictive of the results of long-term carcinogenesis studies, is being addressed by comparably long-term carcinogenesis studies in progress in the collaborative contract program and in the Lung Cancer Branch. In addition, preliminary studies have demonstrated that administration of carcinogens either intratracheally to hamsters in vivo or during incubation of isolated tracheas in vitro resulted in increased thymidine incorporation into tracheal epithelial DNA. Hydroxyurea has been shown in this system to inhibit DNA replication in normal tracheas, as well as in tracheas subjected to physical injury.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the part of the lung cancer program, designed to develop biologic models for the study of respiratory carcinogenesis, its causative factors and host control mechanisms. This assay system permits in vitro identification of critical biochemical and morphologic alterations which occur in the course of respiratory carcinogenesis induced in vivo. The methods developed permit examination of certain aspects of nuclear and cytoplasmic metabolism in viable respiratory epithelial cells. The effects of carcinogens administered in vitro can be examined biochemically. Furthermore, these methods will permit direct biochemical study of a respiratory epithelium during the course of in vivo carcinogenesis. This work coordinates with the in vivo carcinogenesis bioassays being conducted under contracts.

Proposed Course of the Project:

Current studies will be brought to their conclusions and the results will be reported.

Publications

Kaufman, D.G., Genta, V.M., and Harris, C.C.: Studies on carcinogen binding in vitro in isolated hamster tracheas. In Karbe, E. and Park, J. (Eds.) Experimental Lung Cancer. Heidelberg, Springer-Verlag, 1974, pp. 564-574.

6. COLLABORATIVE PROGRAM REPORTS

SUMMARY REPORT

OFFICE OF THE ASSOCIATE DIRECTOR FOR CARCINOGENESIS

July 1, 1974 through June 30, 1975

A group of contracts is funded directly through this Office rather than through the collaborative segment. They include three large programs encompassing many different projects each and, in addition, they include some projects which relate to multidisciplinary efforts not specifically identified with any single segment. Responsibility for technical monitoring is assumed by several Carcinogenesis staff members.

The three large multidisciplinary programs are supported respectively (1) through the NCI program at the Frederick Cancer Research Center managed by Litton Bionetics, Inc. (NO1-CO-25423); (2) through an interagency agreement with Energy Research and Development Administration (YO1-CP-50200) (ERDA formerly Atomic Energy Commission (AEC) for the Oak Ridge National Laboratory, and (3) through the university-based program at the Eppley Institute for Research on Cancer at the University of Nebraska (NO1-CP-33278).

Four research projects are also supported directly from this office. They include the following: a) The development of a liver cell culture system for studies on neoplastic transformation of epithelial cells in vitro by aromatic amine carcinogens (Columbia University, NO1-CP-23234). b) A study of factors that control the growth of cells in culture in relation to transformation by chemical carcinogens, using mouse 3T3 cells (The Salk Institute, NO1-CP-33405). c) A coordinated comparative study of the metabolism of major environmental chemicals and of their activity in mutagenesis and in vitro carcinogenesis tests (International Agency for Research on Cancer, NO1-CP-55630). d) Study of the carcinogenic and other late effects of cancer therapy (radiotherapy and chemiotherapy), second primary cancers in relation to the risk for patients who survive therapy of a first primary, particularly childhood cancers. (Memorial Hospital for Cancer and Allied Diseases NO1-CP-23286).

The three large multidisciplinary programs represent major national research centers for carcinogenesis studies, which bring staff from diverse scientific and organizational backgrounds together into a coordinated national effort in carcinogenesis. Studies are principally aimed at the identification and interpretation of environmental and occupational carcinogenic hazards, integrating basic and applied research methods in the study of carcinogenesis mechanisms and their inhibition.

The Carcinogenesis Program at the Oak Ridge National Laboratory continues to develop an extensive coordinated program in chemical and biological research on carcinogenesis at the animal, cellular, macromolecular, and chemical levels. Its main objectives are to carry out large carcinogenesis

and co-carcinogenesis bioassay studies with particular emphasis on inhalation methods; to develop biological models and refined testing methods for carcinogenesis studies; to define the occurrence, conditions of formation, synthesis, metabolism, and biological activity of N-nitroso compounds, which represent a major class of carcinogens; and to study the molecular target events in DNA damage and repair in relation to chemical and physical carcinogens.

Major findings include the preliminary evidence of a co-carcinogenic effect of the inhalation exposure of NO_2; the elucidation of mechanisms of elution of benzo[a]pyrene from the surface of carrier particles; additional evidence of the role of vitamin A deficiency in the susceptibility to respiratory cancer and of the inhibitory effect of vitamin A administration; the successful development of organ culture systems for respiratory tissues and their application of carcinogenesis studies. Nitrosamine precursors, particularly amino compounds with environmental significance, were tested together with nitrite: carcinogenic effects were found only with some test materials and not with others. Analytical methods for the environmental identification of nitrosamines were developed and studies on the mechanisms of carcinogenicity of N-nitroso.compounds have been further extended. The study of DNA repair mechanisms in carcinogenesis has continued; by using bromodeoxyuridine photolysis, which permits an estimation of the number and size of repaired regions in DNA, chemical carcinogens were classified with respect to the mode of repair induced in human cellular DNA. The development of a method for the neoplastic transformation of fish cells after single exposure to ultraviolet radiation was used to show that subsequent photoreactivation inhibits UV induced transformation. This provides evidence that the initial macromolecular change in DNA is the formation of pyrimidine dimers. This methodology has been extended to chemical carcinogens. Additional studies on the role of membrane transport systems in the growth requirements for transformed cells have been developed.

The Carcinogenesis Program at the Eppley Institute for Research on Cancer at the University of Nebraska continues to develop a combined approach to bioassays of environmental carcinogens and studies on carcinogenesis models and mechanisms. The major finding of a highly effective carcinogen (diisopropanol-nitrosamine) for the induction of pancreatic ductal carcinoma has provided a much needed experimental model for the study of this major form of human cancer. Structure activity relationships of other derivatives of dipropylnitrosamine have carried out to identify the most active compounds in the induction of tumors of the pancreas and/or other sites. The anti-schistosomal drug, niridazole, has been found carcinogenic by feeding to both mice and hamsters. Metabolic and biochemical studies have continued on several classes of carcinogens, with particular emphasis on N-nitroso compounds, polynuclear hydrocarbons and organochlopine pesticides. Pathogenesis studies on several organ sites affected by major routes of exposure to carcinogens have also been further extended.

Office of the Scientific Coordinator for Carcinogenesis at the NCI's Frederick Cancer Research Center (FCRC) - Carcinogenesis research at the FCRC began when the laboratories were opened in June 1972. Four "tasks" were established at that time, (1) Selected Bacterial Species, (2) Large Scale Bioassay,

(3) Preparation and Characterization of Carcinogens, and (4) In Vitro Bioassay. The tasks required a certain amount of collaborative effort, but they were not designed as a unified program. In subsequent contract renewals, the tasks became known as "projects," their functions were modified slightly and their titles changed accordingly to (1) Laboratory Studies of Etiology of Cancer of the Digestive System, (2) Bioassay Research,(3) Preparation and Characterization of Carcinogens, and (4) In Vitro Bioassay Methodology for Potential Carcinogens. In addition, two new projects were added: (1) Procedures for Safe Handling of Chemical Carcinogens, and (2) Chemical Carcinogenesis Research.

During the present year, the Office of the Scientific Coordinator for Carcinogenesis, FCRC, has been the focus of a major planning effort which, through consultation with NCI staff and with advisors from other institutions, has led to the development of a plan for the future development of the FCRC carcinogenesis laboratories. Under this plan, the functions of the present laboratories and other new laboratories to be established in the future will be devoted to one central goal, the development of improved methodology for identification of human carcinogens and for the identification of population groups which are exposed to carcinogens. As resources become available for achieving this goal, a broad approach will be taken including research and development of improved techniques at the molecular, subcellular, cellular and whole animal level. At the present time, there are very few laboratories in the world where all of these approaches to carcinogen identification are undertaken together, thus insuring comparison under controlled conditions.

Presently established laboratories have capabilities in the development of cell culture transformation tests for carcinogenesis, development of better animal bioassay systems, identification of carcinogens of endogenous origin related either to hormonal effects or bacterial metabolism, and analytical chemistry techniques, good instrumentation was assembled to support these projects. In addition, a laboratory is in the beginning stages of development for the study of the critical target sites for carcinogen interaction in cells, as an approach to carcinogen identification. This newest laboratory will require some expansion of personnel as well as provision of adequate laboratory space in order to make it an effective functioning unit in the mission of the FCRC Carcinogenesis Program. Other high priority areas for development include the following: (1) a considerable expansion of the presently very limited capability in organic synthesis in order to provide otherwise unavailable radioactively labeled carcinogens, postulated intermediates of carcinogen metabolism, and other reactive chemicals for study in the overall program; (2) development of a laboratory studying mutagenesis in various bacterial and eukaryotic systems as a technique for identifying potential carcinogens; and (3) the recruitment of personnel competent in studies on metabolic activation of carcinogens for the development of techniques suitable for metabolic activation of carcinogens in conjuction with in vitro test systems.

The present program organization is along the lines of the six projects listed above. It is likely that during the next year, a new plan of organization will be developed in collaboration with the newly recruited FCRC

Carcinogenesis Director, Dr. Charles Irving. The purpose of such a reorganization will be to accomplish more effectively the goals of the new program plan developed for FCRC.

The individual projcets as they have existed during the past year and highlights of their research are listed below:

Laboratory Studies of Etiology of Cancer of the Digestive System - (Project 7) studies the role of materials in the digestive tract on the development of cancer within it. Particular emphasis is on dietary constituents and bile, their metabolism by intestinal tract bacteria, and the role of these materials and their metabolic products on the induction of cancer of the colon and rectum.

The Ames mutagenesis assay has been utilized in this project for screening tryptophan metabolites for mutagenicity and therefore for possible carcinogenicity. Except for a contaminant in one commercial sample, no tryptophan derivatives were found to be mutagenic nor was mutagenic activity found in the filtrates of cultures of intestinal tract bacteria cultured anaerobically with tryptophan.

Work on bile acid metabolism has included the development of improved methods for separation and identification of these materials. Nine of 13 human colonic bacterial species studied were able to hydrolyze the amide bond of the conjugated bile acid taurolithocholic acid under anaerobic conditions. At least two bacterial species (Bacteroid fragilis and Citrobacter sp. were able to form the ethyl ester of lithocholic acid under anaerobic conditions when ethanol was present in the incubation mixture.

Bioassay Research (Project 8) Environmental chemicals are tested for carcinogenicity by administering them to rodents for a major portion of their life span. In addition, research is undertaken to develop improved techniques for the identification of environmental carcinogens. The goals of this research are to develop more reliable assay procedures, to find ways of reducing the cost of detecting carcinogens through bioassays without reducing their reliability, and to find more reliable ways to extrapolate results of these bioassays to the problem of carcinogenicity in man.

Research designed to improve the accuracy of estimation of the maximun tolerated dose (MTD) for long serum administration included the development of a new computerized data system which continuously plots the weight of test animals compared to controls and solves for the dose which will produce a ten percent weight depression. Twenty-one environmental chemicals are currently on chronic bioassay for carcinogenicity.

Preparation and Characterization of Carcinogens (Project 9) This is a resource for the synthesis, analysis, and distribution of chemicals needed by the Carcinogenesis Program. Chemicals being tested for carcinogenicity are analyzed for their purity and stability under conditions of administration in bioassays. Carcinogens which are not commercially available, postulated intermediates in the processing of carcinogens and noncarcinogenic analogs are maintained as samples of known purity in a

repository, for distribution as chemical standards to other laboratories involved in carcinogenesis research.

In Vitro Bioassay Methodology for Potential Carcinogens (Project 10) The methodologies of cell culture carcinogenesis are examined to determine conditions of maximum sensitivity and reproducibility, and to characterize end points to malignant transformation in vitro. A major goal is to standardize such techniques to make them widely useful in many laboratories for the screening or detection of environmental carcinogens.

A standardized hamster embryo cell in vitro carcinogenesis bioassay procedure has been established using aliquot samples of primary embryo cells stored in liquid nitrogen as the source of target and feeder cells. A list of chemicals with known carcinogenic or mutagenic activity was selected and is being used for evaluating the reliability of the system. To date, approximately 40 chemicals have been assayed. These include carcinogenic and noncarcinogenic analogs of some direct alkylating agents, polycyclic hydrocarbons, nitrosamines and nitrosamides, aromatic amines and aminoazo dyes, and a few miscellaneous compounds. Almost complete correlation was found with their reported carcinogenic activity in animal systems. A few procarcinogens such as diethylnitrosamine, N-2-acetylaminofluorene, auramine, and urethan, which require metabolic activation, failed to induce transformed colonies.

Procedures for Safe Handling of Chemical Carcinogens (Project 15) An effort is being developed to help NCI formulate effective measures for the safe handling of chemical carcinogens in the laboratory and their subsequent disposal so that they will not be a hazard to either laboratory workers or to the surrounding community.

Chemical Carcinogenesis Research (Project 16) Three areas of research are under development: (1) immunologic studies on carcinogenesis and the identification of techniques for enhancing immunologic control of developing neoplasias, (2) studies on the interactions between carcinogens and cellular constituents required for neoplastic transformation and the utilization of this knowledge for identification of environmental carcinogens, and (3) investigation of the role of hormones in the induction of carcinogenesis and in determining the susceptibility of hormone-responsive organs to carcinogenic chemicals.

Both estrogen and progesterone receptors have been demonstrated in the mammary epithelial cells of rats which were in a stage of development when tumors can be induced. Furthermore, the cytoplasmic-nuclear transfer of receptor appears to operate in the mammary gland.

Binding of 7,12-dimethylbenz[a]anthracene (DMBA) to the DNA, RNA and protein of mammary epithelial cells was examined. As a control, binding to macromolecules of the liver, which is not susceptible to tumor induction by DMBA, was measured. It was found that the amount of DMBA bound to mammary epithelial DNA varies as a function of the age of the animal. There is a good correlation between the amount of DMBA bound and the incidence of tumors induced. Furthermore, in the 50-day old animal, the amount of DMBA bound to DNA

increases as the dose of DMBA increases. The reported incidence of mammary adenocarcinoma correlates well with these findings. Binding of DMBA to liver DNA is much lower than that to DNA of the mammary epithelium.

There are two major problems which must be solved before the Carcinogenesis Program at FCRC will become fully effective as a major national resource for the development of improved and more economic test systems for carcinogen identification. First, although the staff initially recruited by the contractor has extensive training and experience in a number of areas of research, in-depth experience in chemical carcinogenesis was not present. Thus, there was a lack of guidance for these personnel in designing research of maximum effectiveness for solving problems of carcinogenesis and cancer prevention. Some steps have been taken to overcome this weakness during the past year. Dr. Charles Irving, a well-recognized and respected scientist in the field of carcinogenesis, has been recruited to be Director of the Carcinogenesis Program for the contractor. He will join the staff toward the end of the present contract year. Dr. Douglas Janss was recruited from the National Cancer Institute to join the contractor's staff late in the last contract year. His experience in carcinogenesis of endocrine target organs has led to the establishment of a productive group working on this problem. Dr. Anthony Dipple, a highly qualified investigator of the critical sites of carcinogen interaction within the cell, has recently come from the Chester Beatty Research Institute in England to join the carcinogenesis staff. Other investigators of comparable qualification in carcinogenesis research will be needed to establish the groups working on mutagenesis and carcinogen metabolism as well as to strengthen the organic synthesis activities and some of the laboratories already in existence. The second major problem relates to the physical facilities available for mounting the type of program envisioned in the new plan for the FCRC Carcinogenesis Program. Although funds for the recruitment for the Carcinogenesis Director, for Dr. Dipple, and for supporting personnel for both were included in negotiations of the present contract year which began on June 26, 1974, neither space nor the necessary renovation funds have been assigned for these groups. In addition, space will be needed in order to establish the other groups proposed above, which are essential for a well-rounded program on the development of better methods for carcinogen identification. By the nature of the new program, close consultation and technical collaboration among different research groups will result in a much more effective operation and thus more efficient utilization of NCI funds than will occur if the laboratory groups are scattered in different buildings resulting in less frequent communication. It would be highly desirable to plan to consolidate as many of these laboratories as possible into a single building.

CONTRACT NARRATIVES

OFFICE OF THE ASSOCIATE
DIRECTOR FOR CARCINOGENESIS

July 1, 1974 through June 30, 1975

COLUMBIA UNIVERSITY (NO1-CP-23234)

Title: Development of a Tissue Culture Transformation System for Aromatic Amine Carcinogens

Contractor's Project Director: Dr. I. Bernard Weinstein

Project Officer (NCI): Dr. Stuart Yuspa

Objectives: Epithelial cell cultures from rat and human tissues are being established and utilized to analyze the mechanism of cell transformation by chemical carcinogens. Criteria for assessing the in vitro transformation of epithelial cells are being developed. These epithelial cell systems may also eventually provide a simple in vitro bioassay for chemical carcinogens. The possible significance of activation of latent viral genomes during chemical carcinogenesis is under investigation in rat hepatoma cell cultures.

Major Findings: A major problem which has been encountered is the very long lag (12-32 weeks) and low efficiency of chemical transformation of epithelial cultures. This may in part reflect the lack of early and simple criteria for assessing transformation of epithelial cells. It has been found that morphologic criteria useful for early detection of transformation in fibroblasts do not apply to epithelial cultures. Growth in semisolid media is a useful criterion which correlates with tumorigenicity, but does not occur until several passages after exposure of normal epithelial cells to the carcinogen. Factors that influence growth in agar have been explored to optimize this criterion. Other parameters of transformation applied to fibroblast cultures including saturation density, serum requirement and plasminogen activator (P.A.) synthesis are being evaluated in normal and tumorigenic epithelial cultures. Low concentrations of Dexamethasone (10^{-8}M) reversibly inhibit P.A. synthesis in certain transformed cell cultures. In some but not all cell lines this is associated with a "phenotypic reversion" of other properties of transformed cells. Evidence has been obtained for the control of P.A. synthesis at the translational level. P.A. synthesis is being assessed in normal epithelial cells at early time points after exposure to carcinogens.

In parallel with the above studies certain hypotheses related to the causal mechanism of chemical carcinogenesis are being explored. Oncornavirus production has been detected in epithelial cell lines established from AAF-induced rat hepatomas, methylcholanthrene or asbestos-induced murine carcinomas and a DMBA-induced rat mammary carcinoma. These viruses have been partially characterized but thus far they fail to demonstrate transforming or tumorigenic activity. The contractors have succeeded in isolating mutants of chemically transformed epithelial cells that are temperature sensitive in the maintenance of the transformed phenotype and these are being used to assess the possible role of viral genomes

in maintenance of the transformed state in our cell lines. These mutants display marked changes in cell surface properties at the two temperatures and studies on the biochemical basis for these changes are in progress. Finally a preparative procedure has been developed for obtaining enucleated mammalian cells (cytoplasts) which remain morphologically intact and synthesize protein for at least 12-24 hours. These will be used to analyze certain questions related to nucleus-cytoplasmic interactions in the mechanism of carcinogenesis.

Significance to Biomedical Research and the Program of the Institute: Although more than 80% of human tumors arise from epithelial tissues, most previous tissue culture work related to chemical carcinogenesis (and to oncogenic viruses) has employed fibroblast or embryonic cell cultures. There is a paucity of cloned epithelial cell cultures which can be used for in vitro studies on chemical carcinogenesis. The development of such systems will facilitate studies on the mechanism of chemical carcinogenesis and will also provide rapid and simple bioassays for compounds which may be carcinogenic.

Both RNA and DNA viruses are being detected with increasing frequency in spontaneous, radiation and chemically-induced tumors. Detailed studies are required to determine whether or not these viruses play a causative role in the induction of these tumors, are cofactors in the transformation process, or are merely symptomatic of an aberration in the control of gene expression that characterizes neoplasia.

Proposed Course: Additional epithelial cell cultures will be established and characterized from rat and human tissues, both normal and neoplastic. (1) Attempts will be made to increase the efficiency and diminish the lag which occurs in the in vitro transformation of epithelial cells by chemical carcinogens. (2) The rat hepatoma associated virus (RHAV) will be further characterized biochemically and in terms of biologic activity to determine its possible role in hepatocarcinogenesis. (3) Mutants that are temperature sensitive in the expression of transformation, which recently were obtained from chemically transformed cells, will be analyzed for biochemical parameters essential to the expression of the tumor phenotype.

Date Contract Initiated: April 1, 1972

Current Annual Level: $133,504

ERDA-NCI Interagency Agreement (Oak Ridge National Laboratory) (YO1-CP-50200)

Title: NCI-ERDA Carcinogenesis Program

Contractor's Project Director: Dr. Francis T. Kenney

Project Officer (NCI): Dr. Allen H. Heim

Objectives: The broad objectives are: (1) to carry out large scale cocarcinogenesis studies in exposure chambers with controlled atmospheres containing selected chemical agents to be tested for direct carcinogenic effects on respiratory tissues or for cocarcinogenic effects with known carcinogens, radiation, and

viruses; (2) to bring to bear on problems of chemical carcinogenesis and the diverse scientific talents of the staff of the ORNL's Biology Division, and the scientific and engineering expertise of other ORNL components. A major part of the funding is associated with the first objective.

Specific objectives are as follows: (1) to develop and define animal models for study of the pathogenesis of human lung cancer with emphasis on interaction of exogenous and endogenous factors, using inhalation exposures as well as other methods of respiratory carcinogenesis studies; (2) to investigate the possible relevance of N-nitroso compounds to cancer, involving (a) a study of the formation of nitroso compounds from amines and nitrite in chemical systems and in vivo, (b) development of analytical methods for nitrosatable amines and a survey of their distribution, and (c) studies of the mechanism of action of carcinogenic nitroso compounds by correlation of structure/activity relations with biochemical reactions involved in carcinogenesis; (3) (a) to analyze the mechanisms for repair of damage to the DNA by chemicals or irradiation in mammalian cells and the role of such damage and repair in carcinogenesis and (b) to conduct membrane studies emphasizing energy sources and growth requirements of human transformed cell lines.

As stated above, one of the major objectives is to incorporate the scientific talents of the Oak Ridge National Laboratory staff into the Carcinogenesis Program. The degree to which this has been accomplished may best be realized by presenting the objectives, major findings and significance of each of the particular efforts.

Date Contract Initiated: January 14, 1963

Current Annual Level: $1,216,500

1. Respiratory Carcinogenesis

Specific Project Director: Dr. Paul Nettesheim

Objectives: To analyze cocarcinogenic effects of physical and chemical agents in the induction of lung cancer. Identification and study of those environmental agents (e.g., irritant gases and dusts, infectious microbes) which may increase the susceptibility of respiratory tissues to normally ineffective doses of carcinogen or which may alter the "effective" tumori genic dose by affecting distribution, deposition and/or clearance of inhaled carcinogenic particulates. To study host factors, which affect tumor induction and tumor progression. To identify the major steps in the morphogenesis of lung cancer on the histological and cytological level. To develop new experimental models for co-factor studies.

Major Findings: (1) An inhalation carcinogenesis study is underway to determine whether reactive gases produced during combustion of fossil fuels, namely nitrogen dioxide, formaldehyde, and acrolein, enhance the carcinogenic potency of benzo[a]pyrene (BP) given by intratracheal injection. The time

relationship between carcinogen administration and irritant exposure is being investigated. Preliminary data are available on one of the BP-NO_2 studies suggesting a co-carcinogenic effect of NO_2.

(2) Studies on vitamin A and cancer induction in the respiratory tract indicate that rats maintained on low vitamin A intake have an increased susceptibility to lung cancer induction. The data also suggest that normal vitamin A storage in the liver may not be sufficient to prevent this state of increased susceptibility if vitamin A intake is low at the time of carcinogen exposure. Carcinogen dose studies show that the influence of vitamin A intake on the tumor response is more readily detected at lower carcinogen exposure levels.

(3) An in vitro organ culture system has been established in which the control of cell proliferation and cell differentiation in respiratory tract epithelium can be readily studied. Culture conditions which favor development of either goblet cells, undifferentiated cells or keratin producing cells, have been determined. The rate of epithelial cell proliferation under the various conditions has also been studied.

(4) Studies on clearance of BP attached to carbon particles from the respiratory tract were carried out using radioactively tagged carbon. It was found that, in vivo, the BP is quickly eluted from the surface of small carbon particles (1-5μ), but only very slowly from the surface of large carbon particles (20μ). In the latter case, the carcinogen is cleared at the same rate as the carrier particles. This finding elucidates an important role of carrier particles in respiratory carcinogenesis studies.

(5) The tracheal transplant model has been further refined to carry out localized tumor induction studies with respiratory tract epithelium. Carcinogen dose response studies with BP and 7,12-dimethylbenz(a)anthracene are underway. Early results suggest that the sensitivity of this assay approaches that of the skin carcinogenesis assay.

(6) Another localized tumor induction system was developed in intact hamsters by repeated washing of a predetermined segment of the trachea with carcinogen. This new experimental model will considerably facilitate detailed morphogenesis studies. The first study correlating the histological lesion with the cells exfoliating from the same lesion was performed.

Significance to Biomedical Research and the Program of the Institute:
Epidemiologic data indicate a multifactorial etiology in human lung cancer. This work is a study, using animal models, of the role of inhaled chemicals and of physical and biological agents influencing the respiratory tract in the development of lung cancers. It is expected that this approach will help to define the decisive environmental and host factors in the pathogenesis of lung cancer. The studies are also aimed at elucidation of the morphogenesis of lung cancer and identification of the essential steps in the development of this neoplastic disease.

2. Nitrosamine Carcinogenesis

Specific Project Director: Dr. William Lijinsky

Objectives: To investigate the possible relevance of N-nitroso compounds in human cancer, involving (1) a study of the formation of nitroso compounds from amino compounds and nitrite in vivo, (2) development of analytical methods for nitrosatable secondary and tertiary amines and a survey of their distribution in food and in the environment, (3) investigation of the mechanism of action of carcinogenic nitroso compounds by correlation of structure/activity relations with biochemical reactions involved in carcinogenesis.

Major Findings: (1) Feeding tests of several amino compounds together with sodium nitrite are almost complete. Many of the amino compounds are found in the environment, or in food or are taken as drugs by large numbers of people, and the tests are designed to show whether sufficient nitroso compound is formed in vivo to give rise to a significant number of tumors in small groups of rats. Aminopyrine and heptamethyleneimine, together with nitrite, have induced high tumor incidences. Oxytetracycline, piperidine, chlorpromazine, hexamethylenetetramine, arginine and trimethylamine oxide together with nitrite have not induced a signficant number of tumors, even though all have been shown to give rise to small amounts of nitrosamines at 37°C and at low concentrations in chemical systems. Similar tests of several other amino compounds are still in progress, including carbaryl, lucanthone, tolazamide, methylguanidine, dimethylphenylurea, methapyrilene, dimethyldodecylamine and cyclizine.

(2) A reliable analytical method for identification and estimation of secondary amines in foods and other materials has been developed. A number of foods have been examined by this method, including fish, beverages and canned meats, particularly those treated with nitrite. Similar analysis of tobacco smoke condensate and tobacco is in progress. Methods for determining tertiary amines in foods and other mixtures are under development, but none is yet reproducible.

(3) A large number of structurally related N-nitroso compounds have been tested in rats or mice at the same molar concentration and large differences in potency have been observed, as measured by the "latent period" of tumor formation. In the series of nitrosopiperidines substitution of methyl groups on either or both alpha carbon atoms substantially reduced carcinogenicity. Substitution of hydroxyl in the 4-position gives rise to liver tumors, which are rarely induced by nitrosopiperidine. Chlorine or bromide substitution in the 3,4-positions increases the carcinogenicity greatly (10-20 times) compared with nitrosopiperidine. A carboxylic acid function anywhere in the molecule eliminates carcinogenic activity. Among methylated dinitrosopiperazines, 2,6-dimethyl is much more carcinogenic than the parent compound, and 2-methyl- is almost as active as 2,6-dimethyl. Similarly, 2,6,-dimethylnitrosomorpholine is much more carcinogenic than nitrosomorpholine. On the other hand, nitrosomorpholine-3,3,5,5-d_4 is considerably less carcinogenic than nitrosomorpholine. These results suggest

that a rate limiting step in carcinogenesis by these nitroso compounds is loss of a proton from a carbon atom alpha to the N-nitroso group.

Tests of several nitrosoalkyl ureas and nitrosoalkylcarbamates on mouse skin have shown considerable differences in carcinogenicity, which can possibly be related to known differences in their structure and reactivity. This system might be more suitable for testing similar nitroso derivatives of pesticides and herbicides that are nitrosatable amino compounds (carbaryl, carbofuran, aldicarb, landrin, etc.) that might be widely distributed in the environment. In collaboration with other members of the Biology Division, other biological effects of these compounds are being explored, such as mutagenicity and damage to human DNA in vitro.

Significance to Biomedical Research and the Program of the Institute: The possibility that N-nitroso compounds might be formed in the gastrointestinal tract from ingested amines and nitrites makes this a significant potential carcinogenic hazard for man. Since most of the N-nitroso compounds tested have proved carcinogenic in one or more species, it is unlikely that man will be refractory to them. To assess their significance, more must be understood about the mechanism of formation of N-nitroso compounds from nitrite and amines of different structures to which man might be exposed. The N-nitroso compounds, through studies of their chemistry and of their distribution and metabolism, could help us understand the mechanisms of chemical carcinogenesis.

Proposed Course: In addition to the program outlined above, there is in progress a comparative study of distribution and metabolism of a few selected N-nitroso compounds in several organs of one or more species in which they have organ specific tumorigenic effects.

3. (a) Repair Mechanisms in Carcinogenesis

Specific Project Director; Part (a): Dr. James D. Regan

Objectives: To elucidate the molecular events which occur in human cells when cellular macromolecules such as DNA are damaged by chemical agents. The sequence of repair events, the modes of repair, and the genetic inhibition (due to chemical inhibitors) of repair are the primary objectives of experimental study. The ultimate goal of these experiments relates directly to the practical problems of human exposure to chemical carcinogens and the rule of repair systems in ameliorating the macromolecular damage caused by these agents.

Major Findings: Methodology for repair studies (bromodeoxyuridine photolysis) permits an estimate of the number of average size of the repaired regions in DNA. With this probed it is possible to classify chemical carcinogens with respect to the mode of repair induced in the human cellular DNA. Present findings show that ethylmethane sulfonate, methylmethane sulfonate, nitrogen mustard and propane sultone are ionizing-type agents inducing "short" repair. N-acetoxyacetyl aminofluorene, ICR-170 and certain diaryl acetylenic carbamates are UV-type agents inducing the "long" mode of repair.

Significance to Biomedical Research and the Program of the Institute: Two significanct questions to be answered with regard to the action of chemical carcinogens on human cellular DNA are: (1) what kinds of chemical lesions are induced in the DNA by carcinogens? and (2) what kinds of repair activities occur in the DNA upon induction of these lesions? The methodology (bromodeoxyuridine photolysis) provides a direct answer to the second question. Regarding the first question, an indirect but reasonably accurate answer is obtained by observing the repair sequence induced and comparing it to the repair sequences seen after induction of DNA lesions of whose chemical nature is known such as lesions induced by the ultraviolet or ionizing radiations.

Proposed Course: To continue investigating the classification (UV or ionizing type) of diaryl acetylenic carbamates (kindly supplied by Dr. James A. Miller, McArdle Laboratory, University of Wisconsin). Preliminary evidence suggests that one of these, acetoxysaffrole, is a UV-type agent, similar to N-acetoxyacetoaminofluorene. Studies of K-region and non-K-region benzyprene epoxides (supplied by the National Cancer Institute) are in progress.

The long-range goals are to sample several chemical carcinogens from all of the chemical classes of carcinogens to determine if the two-repair mode dichotomy maintains or if further, more complex repair systems emerge.

3. (b) Repair Mechanisms in Carcinogenesis

Specific Project Director, Part (b): Dr. John S. Cook

Objectives: To define the relationship between the growth requirements of transformed or nontransformed cultured cells and the role of membrane transport systems in supplying and/or regulating the supply of essential metabolites to meet these requirements; to define the cellular response to attack on transport systems by specific ligands.

Major Findings: Non-growing skin cells transport and back-leak alkali cations slowly. When the cells are stimulated to grow, both processes are approximately tripled in minutes and continue at rates proportional to cell surface area throughout the cell cycle and until growth ceases; cell K:Na:protein ratios remain constant. Alkali cation transport is thus a code terminant (together with membrane growth) rather than a primary stimulus to growth. Na-K transport enzyme turns over 3x faster than bulk membrane protein; the latter is slowest in G1. Cells are derepressed in glucose-poor media to double glucose transport capacity and to depress lactate excretion without an accompanying increase in specific binding of the inhibitor phloretin.

Significance to Biomedical Research and the Program of the Institute: Current thinking about tumorigenesis emphasizes the altered state of the surface membrane, such alterations including changing (usually enhanced) activity of transport systems. This program is concerned with (a) the nature of the enhancement, whether a genetically regulated increase in numbers of transport molecules or an increase in their specific activity; and (b) the physiological role of enhanced transport in relation to growth and growth control, and

whether such enhancement is primarily or secondarily related to sustained growth in transformed cells.

Proposed Course: The relationship of sugar transport to growth control in human cells as well as in chemically transformed rodent cells will be pursued. Special emphasis will be placed on numbers, and the control of these numbers, or transport molecules as assayed by specific ligand binding. Coupling of nonelectrolyte transport to electrolyte metabolism will be investigated by means of potential-sensitive fluorescent dyes.

3. (c) Repair Mechanisms in Carcinogenesis

Specific Project Director, Part (c): Dr. Richard B. Setlow

Objectives: To identify specific ultraviolet induced changes in DNA that are responsible for neoplastic transformation.

Major Findings: Initial experiments show that single exposures of fish cells to UV radiation result in neoplastic transformation, and that if the UV irradiation is followed by photoreactivating illumination, many fewer transformants arise. It is concluded that the initial change in macromolecules resulting from the UV irradiation has been in DNA, and moreover that the particular change in DNA has been the formation of pyrimidine dimers. Fish cells are also transformed by N-acetoxy-AAF with an efficiency predictable from repair studies.

Significance to Biomedical Research and the Program of the Institute: These data permit an identification of the sensitive molecules and the nature of the lesions in them. Hence one will be able to estimate quantitatively the probability of a transformation per individual chemical change in DNA and therefore the quantitative hazards from chemical carcinogens.

Proposed Course: (a) To complete the histological examination of the many fish (P. formosa) injected with cells treated with N-acetoxy-AAF, UV-radiation, or x-radiation; (b) to use the data in (a) to obtain initial dose response curves for eoplastic transformation; (c) to use the fish colony to obtain quantitative data relating the number of physical or chemical damages per genome to eoplastic transformation. (d) to establish an in vitro cell culture system of P. formosa cells. Such a system will simplify both chemical and physical dosimetry and make it possible to determine, easily, molecular changes in DNA associated with the transformation process as well as the effects of inhibitory agents on the process. The in vitro grown cells will be used as a cross check on the results obtained with cells grown in vivo.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-55630)

Title: Evaluation of the Significance of Experimental Chemical Carcinogenesis Data to Man

Contractor's Project Director: Dr. Lorenzo Tomatis

Project Officer (NCI): Dr. John A. Cooper II

Objectives: The goal of this research activity of the International Agency for Research on Cancer (IARC) is the development of objective criteria for the extrapolation of animal data to the human situation. This would allow for the identification in a straightforward manner of those chemical exposures in the environment of man for which substantive risk exists. Only in such a context can meaningful risk-benefit evaluations be carried out. To assess the significance of experimental data to man, the research focuses on three major topics: (1) comparative metabolism of carcinogens in human and animal systems; (2) chemical carcinogenesis in vitro; and (3) mutagenesis Testing.

Major Findings: (1) Comparative metabolism of carcinogens in human and animal systems--The known mammalian metabolites of DDT in rats or mice are being investigated for their pathways of enzymatic degradation or metabolic activation using in vitro techniques. The synthesis of possible reactive intermediates of these compounds is under way. Investigations of the biotransformation and comparative metabolism of vinyl chloride and a number of nitrosamines are being conducted in human and animal tissues. Characterization of the binding sites of vinyl chloride metabolites with nucleic acid bases is under way. Studies of the stability of alkylated nucleic acid bases in target and nontarget organs are being carried out in the context of nitrosamine carcinogenesis.

(2) Chemical carcinogenesis in vitro--Studies on the isolation and single cell cloning of epithelial and mesenchymal cells from human and rat liver, as well as morphological and biochemical characterization of these cells, are under way. The rat liver epithelial cells are being exposed to a variety of carcinogenic substances (nitrosamines, aflatoxins, polyaromatic hydrocarbons, and vinyl chloride) and the transformed cells have shown the development of carcinomas following back transplantation in the first series of experiments. Similar studies are under way with human liver cells and the malignant potential of transformed clones is being tested by transplantation into nude mice.

(3) Mutagenesis testing--In vitro mutagenicity testing has been used in studies with vinyl chloride. It has been demonstrated that liver tissue of humans, rats, and mice were effective in converting the compound into highly mutagenic metabolites. The metabolic activation of dimethyl- and diethylnitrosamine in rat and hamster tissues has also been investigated using tissue slice techniques and mutagenesis assays. The results indicate that the organ distribution of tumors following the administration of these substances is greatly dependent on the capacity of an organ to metabolize the compounds to electrophylic forms.

Significance to Biomedical Research and the Program of the Institute: These studies are designed to provide a context in which meaningful risk-benefit evaluations for man could be carried out.

Proposed Course: The activity will continue for the next two years along the same general lines indicated in the Major Findings.

Date Contract Initiated: September 1, 1974

Current Annual Level: $134,900

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASE (NO1-CP-23286)

Title: Oncogenesis and Other Late Effects of Cancer Therapy

Contractor's Project Director: Dr. Giulio J. D'Angio

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: (1) Determination of the "spontaneous" incidence of second primary tumors in patients with Wilms' tumor, neuroblastoma, and soft tissue sarcomas by chart reviews of short-term survivors; (2) determination of the incidence of "induced" second primary tumors by chart reviews of long-term survivors; (3) examination of long-term survivors, especially those considered at high risk as a result of the analysis in number 2 above, to detect maligant and possibly premalignant conditions; (4) analysis of patients with second malignant neoplasms (SMNs) by type and number to detect possible correlations between age, sex, primary tumor diagnosis, genetic predispositions, familial clusters, treatments given, and latent periods; and (5) establishment of a center repository of unusual problems of childhood cancer.

Major Findings: (1) The pilot study at Children's Hospital of Philadelphia of long-term survivors at particular risk for developing organ damage, was completed. It revealed that physical examination alone is inadequate to detect organ malfunction. Many patients treated with radiation, with or without chemotherapy, had some degree of renal impairment (10/50) and pulmonary dysfunction (9/50) without physical or roentgen findings. No specific association between second malignant neoplasms and organ damage was detected in this group of intensively treated survivors. (2) Hospital chart review of long-term survivors of childhood cancer was conducted at ten institutions. Analysis of the data was made relating recurrence of second malignant neoplasms and several variables. The data suggest there are at least two patient populations: children with second malignant neoplasms appearing within five years of the time of diagnosis, and those developing such tumors thereafter. The first group might be considered "spontaneous"; the second, more probably related to treatment. It was found that irradiated children receiving actinomycin D (AMD) had a decreased risk for developing SMNs by a factor of seven. The "protection" afforded by AMD was dynamic; that is, children receiving repeated courses of AMD had fewer tumors than those receiving one or two courses. This study was conducted by the case-control method of analysis.

Significance to Biomedical Research and the Program of the Institute: Chemotherapy, combined with surgery and radiation for the treatment of children with Wilms' tumor and soft tissue sarcomas, has resulted in significantly improved cure rates of these diseases. It is essential that the risks inherent in these treatment regimens for both organ damage and carcinogenesis be understood by physicians who follow these children. Methods for periodic examinations have been delineated by the study. Knowledge of the etiologic mechanisms of oncogenesis will be expanded by collection of data regarding

SMNs in this group of treated survivors. Ascertaining the incidence of second cancers in patients not subjected to radiation and chemotherapy, in patients with congenital malformations and familial diseases, as well as that associated with specific treatments, is of concern. The finding that AMD decreases the frequency of radiation-associated tumors has both laboratory and clinical implications. The interaction of AMD and radiation is well-known; a better understanding of radiation oncogenesis may be afforded by detailed study of the actinomycin D/radiation treatment interaction. A delineation of the frequency of SMNs in long-term survivors of childhood cancer may permit identification of patients at high risk for this complication.

Proposed Course: (1) SMN data provided by the ten cooperating institutions will undergo continuing analyses to detect possible correlations between primary and second malignant neoplasms, age, sex, latent periods, and possible associations between the type of primary and second malignant neoplasms. (2) A work party will screen proposals submitted by candidates for establishing a central repository of unusual problems of childhood cancer. Representatives of interested institutions will meet in Philadelphia in May to consider the proposals, and to choose among them. (3) Chromosomal analyses of selected patients derived from the Children's Hospital of Philadelphia will continue. Subjects will be patients with identifiable abnormalities; some patients are still on therapy, others have come off treatment recently. The examinations will seek to determine which of the changes are attributable to therapy and if so, which type, and whether the abnormalities are reversible. (4) Long-term survivors will continue to receive periodic examinations in the effort to detect early signs of somatic and psychic damage. (5) It is proposed that a laboratory investigation be undertaken to determine whether AMD protection of RT-associated malignant neoplasms can be substantiated in the laboratory, and possible mechanisms be explored in animal models.

Date Contract Initiated: June 26, 1972

Current Annual Level: $74,607

NCI, FREDERICK CANCER RESEARCH CENTER (LITTON-BIONETICS, INC.) (NO1-CO-25423)

Title: Operation and Maintenance of the NCI's Frederick Cancer Research Center

Contractor's Project Director: Dr. Robert E. Stevenson

Project Officer (NCI): Dr. William W. Payne

Carcinogenesis Coordinator (NCI): Dr. Richard R. Bates

Objectives: At the Frederick Cancer Research Center (FCRC) there is a diverse program of research and resource production in collaboration with the various divisions of the National Cancer Institute. Approximately 35% of the activities are supported by the Carcinogenesis Program. Historically the research in chemical carcinogenesis was organized as several separate projects, each with its own distinct goals, and each managed by a senior scientist on the contractor's staff and monitored by a member of the NCI staff. Although the organizational plan still maintains these individual projects, the entire FCRC carcinogenesis

program is now in a transition period toward a more unified research plan. The central purpose of this plan is to utilize observations of basic carcinogenesis research to develop improved methods for detecting carcinogens capable of inducing cancer in humans. The methods will be ones appropriate for the identification of exogenous carcinogens present in the human environment and also for identification of endogenous carcinogens which are formed de novo within the body. Approaches will include identification of molecular interactions characteristic of carcinogens, mutagenesis screens, in vitro transformation of mammalian cells and improved in vivo bioassays using whole animals. The individual projects as they currently exist are discussed below.

PROJECT 7

Title: Laboratory Studies of Etiology of Cancer of the Digestive System

Contractor's Project Manager: Dr. Milton Slein

Project Monitor (NCI): Dr. Sidney Silverman

Objectives: The objectives of Project 7 are to investigate the metabolism of bacterian indigenous to the human colon in order to determine their abilities to produce carcinogens, carcinogen precursors or co-carcinogens, and to identify such compounds chemically. From the results of epidemiological, clinical and laboratory studies by others, it was decided to initiate tests with tryptophan and steroids as putative agents of colonic cancer mediated by bacteria.

Major Findings: The Ames mutagenesis assay has been utilized in this project for screening tryptophan metabolites for mutagenicity and therefore for possible carcinogenicity. Except for a contaminant in one commerical sample, no tryptophan derivatives were found to be mutagenic nor was mutagenic activity found in the filtrates of cultures of intestinal tract bacteria cultured with tryptophan. Bacteria from rats on an all beef diet were also incubated anaerobically with tryptophan or various of its derivatives including anthranilic acid, 3-hydroxyanthranilic acid, kynurenine, 3-hydroxykynurenine, kynurenic acid, quinaldic acid, and xanthurenic acid. There was no mutagenic activity from any of these filtrates either, though one incubation mixture consisting of several different derivatives inhibited the mutagenic activity of 2-aminoanthracene in the presence of liver microsomes. Work on bile acid metabolism has included the development of improved methods for separation and identification of these materials. Nine of 13 human colonic bacterial species studied were able to hydrolyze the amide bond of the conjugated bile acid taurolithocholic acid under anaerobic conditions. At least two bacterial species (Bacteroids fragilis and Citrobacter sp. were able to form the ethyl ester of lithocholic acid under anaerobic conditions when ethanol was present in the incubation mixture. Long-term experiments are under way comparing the tumor incidence in rats on normal lab chow with those on an all beef diet. Groups on both diets are also given intrarectal treatment with methylnitrosonitroquanidine to study the possible co-carcinogenic activity of the diets.

Significance to Biomedical Research and the Program of the Institute: There is epidemiologic evidence suggesting that a high incidence of colon cancer is

associated with a high meat diet. Since the epithelium of the colon is in constant contact with anaerobic bacteria, it seems likely that their metabolic activities may be of considerable significance in determining the levels and types of carcinogens to which the epithelium is exposed. This project examines the bacterial metabolism of classes of chemicals now considered to be among the potential substrates for carcinogen production.

Proposed Course: In the absence of any positive results for mutagenicity of tryptophane derivatives, this portion of the study will be concluded during the next few months. Work will continue on bile acid metabolism and the search for carcinogens or co-carcinogens among bile acids. In addition, it is planned to investigate the presence of enzymes related to the activation and inactivation of known chemical classes of carcinogen among the anaerobic bacteria of the intestinal tract.

PROJECT 8

Title: Bioassay Research

Contractor's Project Manager: Dr. Elliot Gordon

Project Monitor (NCI): Dr. Thomas Cameron

Objectives: The primary objectives of the Bioassay Research Project are: The conduct screeing studies of selected environmental chemicals to determine their carcinogenic potential in Fischer rats and $B_6C_3F_1$ mice; to conduct research to improve the protocols under which these studies are conducted; and to provide pathology support for these studies, for other projects at FCRC, and for collaborative research with other laboratories.

Major Findings: Twenty-one environmental chemicals are now on test in chronic bioassay in rats and mice. These represent the first bioassays performed in this facility and it will be several months before the first of these reach their completion date. Dosage level determination for these compounds in rats was remarkably effective from a 12-week subchronic study. With mice, however, the determination of maximum tolerated dose was much less accurate and dosages have had to be adjusted on ten chemicals some weeks after beginning the chronic studies. Because of this inaccuracy, a 26-week subchronic study in mice is under investigation to try to improve the accuracy. Other activities designed to improve the accuracy of estimation of MTD have included the development of a new data system which continuously plots the weight of test animals compared to controls and solves for the dose which will produce a ten percent weight depression. Collaborative studies under way with other FCRC projects include investigation of changes in serum haptoglobin levels associated with tumor induction, the effect of an all meat diet on tumor development in rats and on the susceptibility of the colon to induction of tumors by methylnitrosonitroguanidine, and the susceptibility of various strains of rat to induction of mammary tumors by 7,12-dimethylbenz[a]anthracene.

The pathologists, in addition to supporting this project, have supported the animal farm and other collaborative projects both by offering histopathological

support and contibuting to experimental design.

Significance to Biomedical Research and the Program of the Institute: In addition to its role in testing environmental chemicals for possible carcinogenicity, this project is a major resource to NCI for the development of improved methodology for the bioassay of chemicals.

Proposed Course: This project will continue to investigate the carcinogenicity of environmental chemicals by standard NCI protocols. In addition, they will undertake investigations designed to improve the nature of these protocols and to provide more meaningful ways to extrapolate the results of animal experiments to the problem of human carcinogenicity.

PROJECT 9

Title: Preparation and Characterization of Carcinogens

Contractor's Project Manager: Dr. Walter Zielinski

Project Monitor (NCI): Dr. Marcia Litwack

Objectives: This project provides analytical and synthetic chemistry support to other projects at FCRC and to cancer research laboratories elsewhere throughout the world. New analytic techniques are developed as needed. The bulk of the analytic activity is in relation to the bioassay program for which chemicals are procured, analyzed for purity, stability, and homogeneity within feed mixtures. Feed and bedding are analyzed for pesticide and mycotoxin residues. Crude chemicals are purified when necessary for program purposes. The analytic resource is also used for detecting and identifying metabolites of exogenous chemicals or biologic materials. Carcinogens, their analogs, and metabolic products are synthesized when suitable materials cannot be obtained commerically. The project also maintains a chemical repository for storage and distribution of chemical carcinogens and their analogs for reference purposes. The metabolism of nitrosamines in relation to carcinogenesis is under investigation.

Major Findings: The major activities of this project include analysis of the purity and stability of chemicals and the homogeneity of feed and other mixtures containing test chemicals. The greater portion of this work supports the bioassay research project, but analyses are also done for other projects at FCRC. Analytical methods available include the following: (a) GLC-mass spectrometry for selective ion monitoring, establishment of a graphic display data computer accessory, sensitivity enhancement, applications to the analysis of carcinogens in biological media including blood clearance kinetics of dimethylnitrosamine, quantitative MS analysis; (b) IR technique for microsampling, FMIR analysis, low temperature spectroscopy, structural interpretations; (c) atomic absorption spectrometry for trace metals in biological systems, improvement of graphite tube accessory; (d) establishment of high resolution FT-NMR capabilities for ^{1}H and ^{13}C spectroscopy; (e) TLC development for quantitative data automation, component isolation, preparative TLC,

rapid solvent selection; (f) automated quantitative elemental analysis for S and O as well as CHN; (g) DSC development for phase transition studies; (h) high pressure LC development for automated quantitative purity analysis, fraction collecting, gradient analysis, mutliple wavelength detection, application to the separation of nucleic acid bases; GLC development for the use of liquid crystals for separation of polyaromatic hydrocarbons, establishment of an unattended overnight GLC and data analysis system, development of protocols for evaluation for chemical-feed blending homogeneity studies, support analysis for synthesized organics, development of procedures for organic trace and analysis and derivative preparation.

Other activities have included (1) biochemical confirmation of the deuterium isotope effect in vivo methylation of rat liver, DNA, RNA, protein and of the N-7 position of guanine in nucleic acids and the observation of this effect in an analytical study of blood clearance kinetics, in studies of the metabolism of dimethylnitrosamine; (2) the establishment and operation of the NCI Carcinogenesis Reference Compound Bank in filling 71 continental and 9 international requests for carcinogen standards, including the development of controlled chemical storage and safe handling protocols for 73 current standards (over 4600 units), chemical subdivision capabilities for small units, and considerations for effective and safe operation of a Chemical Repository; and (3) the initiation of organic synthesis, including the planned development of a synthetic area for radiological and carcinogen synthesis.

Significance to Biomedical Research and the Program of the Institute: Adequate bioassay research can only be done with proper analytic chemistry backup for the identification of chemicals being administered, analysis of their purity, and indication of the length of time they are stable under conditions of storage and administration. This project provides that type of support. In addition, it is a central distribution point for the supply of reference standards on carcinogenic chemicals and their analogs for laboratories throughout the world. An expanding activity of this project is the synthesis of chemicals needed to study the metabolism of carcinogens, or the relationship between chemical structure and carcinogenic activity. It is anticipated that in the future the project will develop the capability for synthesizing radioisotopically labeled chemicals for laboratory investigation. This is a resource which is useful to the Carcinogenesis Program both at Frederick Cancer Research Center and elsewhere throughout the world.

Proposed Course: A major activity will continue to be in analytic chemistry, both as support for Project 8 and also increasingly to investigate metabolites of interest to other laboratories in the Carcinogenesis Program. Synthetic activities will increase and the capability of synthesizing isotopically labeled chemicals will be developed. An increasing proportion of the effort will be devoted to the development of prescreens for carcinogens based on principles of organic and analytic chemistry.

PROJECT 10

Title: In Vitro Carcinogenesis

Contractor's Project Manager: Dr. Roman Pienta

Project Monitor (NCI): Dr. Allen Heim

Objectives: The objective of this project is to develop standardized in vitro bioassay methods for detecting potential carcinogens which may present a hazard for man. Emphasis is placed on defining standard experimental conditions and end points which are reproducible, rapid, and valid for detecting potential human carcinogens.

Major Findings: A standardized hamster embryo cell in vitro carcinogenesis bioassay, using aliquot samples of cells stored in liquid nitrogen as the source of standard target and feeder cells, has been established. A list of standard cheimcals with known carcinogenic or mutagenic activity was selected and is being used for evaluating the reliability of the system. To date, approximately 40 chemicals have been assayed. These include carcinogenic and noncarcinogenic analogs of some direct alkylating agents, polycyclic hydrocarbons, nitrosamines and amides, aromatic amines and aminozao dyes, and a few miscellaneous compounds. Almost complete correlation as to their reported carcinogenic activity in animal systems was found. A few procarcinogens such as diethylnitrosamine, N-2-acetylamino fluorene, auramine, and urethan, which require metabolic activation failed to induce transformed colonies.

Studies concerning the metabolic activation of procarcinogens in a completely in vitro system by using activated liver microsomes are in progress. Preliminary data indicate that N-2-acetylaminofluorene can be activated.

Various criteria for in vitro transformation by chemical carcinogens are being investigated. The ability of transformed hamster cells to grow in semisolid agar correlates with tumorigenicity in nonimmunosuppressed hamsters.

Human cells previously treated with a chemical carcinogen and capable of three dimensional colonial growth in agar have been isolated and are being further characterized.

Significance for Biomedical Research and the Program of the Institute: Increasing numbers of chemicals are being introduced into use each year thereby increasing the number of potentially carcinogenic chemicals which should be tested. In vivo testing is costly and time consuming. Consequently, the development of a rapid, relatively inexpensive, reliable in vitro test for carcinogens would provide a system for prescreening chemicals to select those most in need of chronic testing in vivo. Use of the prescreen introduces a much needed method for improving the selection of chemicals entered into the costly in vivo bioassay system thereby improving the efficiency and overall effectiveness of the entire carcinogen detection system.

Proposed Course: Efforts will continue on identification of experimental conditions leading to maximum susceptibility to transformation by chemical carcinogens by both human cells and cells of experimental animals. Transformation by a broader range of classes of chemical carcinogens will be investigated and effort will be applied to coupling the in vitro system with suitable chemical metabolism systems to activate carcinogens which are not directly carcinogenic.

PROJECT 12

Title: Production of Inbred and Hybrid Laboratory Animal Strains

Contractor's Project Manager: Dr. Raymond Ediger

Project Monitor (NCI): Dr. William Payne
Dr. Thomas Cameron

Objectives: To provide for production of inbred, hybrid and outbred laboratory rodents of various species and strains, in healthy condition and of known genetic background.

Major Findings: The animal farm provides high quality laboratory animals for a variety of programs within NCI. During the calendar year 1974, over 433,000 animals of 26 different species or strains were produced for use in cancer research programs throughout the country. Approximately 35 percent of the program is supported and utilized by the NCI Carcinogenesis Program. The bulk of the output for this program is in Fischer/344 rats and $B_6C_3F_1$ hybrid mice, animals used in the standard NCI bioassay protocol. This is the major source of these animals used in bioassay contracts throughout the country. Several other strains of mice, including the nude athymic mouse, are also produced as are three strains of guinea pigs and one of rabbit. In addition to the production of young adult animals, timed pregnancies, newborn, and aging animals have also been supplied. The animal farm has been able to meet all needs of FCRC laboratories for these strains and also to supply significant numbers to NIH intramural laboratories and NCI contractors.

Significance for Biomedical Research and the Program of the Institute: Biomedical research of the scope and the quality envisioned for FCRC must be solidly based upon a resource of various animal species of the highest quality of health and genetic integrity. The FCRC Bioassay task, much of the NCI intramural research and some of the collaborative effort have been given continuity by their use of the animals produced by the FCRC animal farm with the cooperation and direction of the NIH in-house animal genetics unit.

Proposed Course: This project will continue to supply most of the animals required for the Carcinogenesis Bioassay Program as well as significant numbers of animals for other intramural and collaborative carcinogenesis programs. Special emphasis will be placed on meeting the needs of all research projects at FCRC.

PROJECT 15

Title: Procedures for Safe Handling of Chemical Carcinogens

Contractor's Project Manager: Dr. Eric Sansone

Project Monitor (NCI): Dr. Larry Keefer

Objectives: The hazards associated with standard chemical laboratory procedures when working with chemical carcinogens are being evaluated. All available data

bearing on this subject will be gathered and, where necessary, laboratory studies to assess risks will be carried out. Safety protocols for use by laboratory workers handling carcinogens will be developed.

Major Findings: Since early November, when the project acquired a full-time manager, the aim of the program has been to assist NCI in the formulation of effective measures to prevent cancers of occupational origin in laboratory workers and to protect the general environment during research programs involving chemical carcinogens. This goal is being pursued by gathering pertinent information through literature searches, other information retrieval mechanisms, conferences, and visits to contractor and other facilities. Relevant information will be used to revise existing guidelines and to prepare new ones.

Significance to Biomedical Research and the Program of the Institute: Numerous laboratories throughout the world are working with chemical carinogens. Until now, there has not been a central laboratory for the purpose of developing and analyzing safety techniques for the protection of carcinogenesis research workers and the surrounding community from hazardous exposure to chemical carcinogens. This project is designed to meet this need.

Proposed Course: A permanent project manager has been hired. The project has begun by reviewing known approaches to carcinogenesis laboratory safety. Subsequently, necessary research projects will be undertaken.

PROJECT 16

Title: Chemical Carcinogenesis Research

Contractor's Project Manager: Dr. Douglas Janss

Project Monitor (NCI): Dr. Richard Bates

Objectives: This project covers performance of basic research pertaining to carcinogenesis by chemical and physical factors or the prevention of cancer by these agents. The research objectives reflect those areas of science investigation that require a special emphasis or expanded activities and that can maximally utilize FCRC facilities. Such areas may include studies in bioassay, biological models, immunology, molecular biology, and specific organ research.

Major Findings: Antigenicity has been established for six Strain 13 guinea pig tumors. Based upon information obtained from these experiments, a relationship between the antigen city of a tumor and its susceptibility to the anti-tumor activity of BCG will be determined. A guinea pig model for BCG-immunotherapy of hematogenous metastases is being developed. Four murine fibrosarcomas have been characterized for antigenicity. Established fibrosarcoma transplants have been treated by intra-tumor inoculation of BCG and Listeria monocytogenes. Data from these experiments may permit correlation between tumor antigenicity and susceptibility to immunotherapy with these infectious agents. The effect of neonatal injection of BCG on leukemogenesis in AKR and C58 mice is being evaluated.

A morphological study of mammary growth and differentiation was started to serve as a base upon which the examination of the influence of age on the susceptibility of the mammary gland to polycyclic hydrocarbon-induced tumorigenesis could be built. Animals of ages with high, intermediate and low susceptibility to mammary carcinogenesis will be examined. Comparisons are being made using high resolution light and electron microscopy, measurement of alveolar and ductal density, mitotic rate and DNA synthesis rate as measured by ^{3}H-thymidine incorporation. In addition to possible morphological changes with age, the sensitivity (ability of the gland to respond in terms of growth rate, protein synthesis, etc.) of the mammary epithelium to hormones may change as the animal ages. It is evident that the sensitivity of a tissue to praticular steroid hormones depends at least in part on the presence (possibly amount) of a specific receptor for that hormone. At present, there is not information in the literature on progesterone receptors in the mammary gland and few reports of estrogen receptors. Reports of estrogen receptors utilized whole gland (fat and connective tissue as well as epithelium) which reduces the ability to isolate receptor and stimulated glands from pregnant or lactating rats which represent states when the gland is not responsive to tumor induction. Both estrogen and progesterone receptors have been demonstrated in the mammary epithelial cells of rats which were in a stage of development when tumors can be induced. Furthermore, the cytoplasmic-nuclear transfer of receptor appears to operate in the mammary gland. Binding of DMBA to the DNA, RNA and protein of mammary epithelial cells were examined. As a control the binding to liver (which is not susceptible to induction of tumors by DMBA) macromolecules was measured. From these experiments it was found that the amount of DMBA bound to mammary epithelial DNA varies as a function of the age of the animal. There is a good correlation between the amount of DMBA bound and the incidence of tumors induced. Furthermore, in the 50-day-old animal, the amount of DMBA bound to DNA increases as the dose of DMBA increases. The reported incidence of mammary adenocarcinoma correlates well with these findings. Binding of DMBA to liver DNA is much lower than that to DNA of the mammary epithelium which is susceptible to its carcinogenic action. Liver binding appears to vary only as a function of the dose administered. The purification and analysis of DMBA bound to the RNA and protein of the mammary gland and liver is now under way. Experiments have been designed to examine tumor incidence in several rat strains following the administration of DMBA. Three strains (Sprague-Dawley, Long-Evans, and Fischer 344) have been placed on experiment. The remaining strains will be started as soon as adequate numbers can be bred or obtained from outside sources. In addition to the tumor studies, comparisons of possible differences in growth and differentiation, as detected by morphology, mitotic index, DNA synthesis and hormonal sensitivity, is being undertaken.

A murine cell line (238) was transformed following infection of the cells with UV-inactivated herpes simplex virus type 2. The transformed parent cell line (H238) produces tumors with a 100 percent incidence in fully immunocompetent BALB/c mice inoculated with 10^{6} cells. Indirect immunofluorescence tests revealed the presence of HSV specific antigens in the transformed cells. HSV.2 specific antibodies, capable of neutralizing HSV.2 but not HSV.1 virus infections, were found in sera from mice bearing tumors induced by the transformed cells. The transformed cells do not harbor infectious HSV.2 particles,

nor could infectious HSV DNA be rescued. In the same model system the enhancement of transformation by herpes simplex virus (HSV) with certain chemical carcinogens was studied. Polycyclic aromatic hydrocarbons, i.e. DMBA, were used to pretreat primary BALB/c embryo (BE) cells and normal human fibroblast (HF) cells prior to infection with HSV. Cultures were then monitored for transformation. General characteristics of this system may provide insight and reagents for evaluating the relationship of HSV.2 to certain forms of human cancer.

Fluctuations of serum haptoglobin (Hp) concentrations in response to transplanted and chemically-induced tumors are being studied. Serum Hp concentrations were determined daily over the entire course of development of five transplanted tumor lines. All mice exhibited elevated Hp levels at the same point during tumor development. However, both the time and extent of the elevation varied considerably, even between closely related tumor lines. Studies of the Hp response to tumors induced with dimethylbenzanthracene and diethylnitrosamine are in progress. The objective of these experiments is to determine the potential utility of Hp as a serum marker for tumor induction in laboratory animals. Further experiments are in progress to study the mechanism of the Hp response to tumors.

Significance to Biomedical Research and the Program of the Institute: This project is undertaking a number of important problems including definition of the conditions under which mammary tissue is susceptible to malignant transformation and the role of hormones in this process, investigations of procedures useful for enhancing the immune surveillance against tumors and the process of carcinogenesis, studies of the interactions of chemical carcinogens and viruses in tumor induction, and markers of the development of early neoplasia under experimental conditions.

Proposed Course: The research discussed above will continue along the lines outlined. In addition, new investigations will be started to define the critical molecular target sites for carcinogen interaction in order to use this information for the development of better approaches to carcinogen identification.

Date Contract Initiated: June 26, 1972

Current Annual Level:

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH ON CANCER) (NO1-CP-33278)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. David B. Clayson

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The bioassay of suspected chemical carcinogens and definition of the biological and chemical mechanisms of the carcinogenic process are

two principal research aims under this contract.

Major Findings: 1. Bioassay--Numerous compounds are being tested in several species. Compounds are selected for bioassay on the basis of their carcinogenic potential, as suggested by physical or chemical properties and structures, relevant known biological effects, or by epidemiological findings which indicate environmental exposure to the compounds presents a high cancer risk. The level of use by the public of these suspect chemical agents is considered in the selection process. An experimental design is developed, based on consideration of present knowledge and the compound's properties. A guiding principle of experimental design is the desire for maximum ability to extrapolate results of each study to man A general policy of collaborative research prevails throughout, utilizing the expertise of chemists, biologists, pathologists, and biochemists in implementing portions of many experiments and in elucidating results.

Highlights of selected bioassay studies are as follows: (a) Suitable animal models for investigation of pancreatic ductal carcinoma, an increasingly important tumor in man, were not available in the past. However, in the course of investigating the biological effects of dipropylnitrosamine and some of its possible metabolites, it was found that 2.2 -dihydroxy-di-n_-propylnitrosamine induced pancreatic cancer in about 80 of the treated hamsters as early as 15 weeks after initiation of treatment. The morphological appearance and biological behavior of these neoplasms resembled those occurring in man. (b) The antischistosomal drug, niridazole, has been tested for carcinogenicity in mice and hamsters. Continuous feeding in the diet to mice led to a lighly significant increase in the number of papillomas and carcinomas of the forestomach and adenomas of the lung, compared to untreated animals. In addition, six bladder tumors were found. Forestomach papillomas were also induced in hamsters. (c) The transplacental exposure of hamsters to ethylurea and sodium nitrite, precursors of ethylnitrosourea, leads to development of neurogenic tumors, principally of the peripheral nervous system in offspring. In contrast, adult animals develop five types of tumors (vascular tumors of the liver and spleen, squamous cell papillomas of the forestomach and vagina, ovarian tumors and neurinomas of the peripheral nervous system). When treated with these precursors, the transplacental system provides a model for von Recklinghausen's disease, with tumors in both man and hamster having a shorter latency in females compared to males. Gonadectomy of the transplacentally treated offspring indicated that these tumors may be inhibited in hamsters by humoral levels of androgens. (d) The incidences of spontaneous neoplastic and non-neoplastic diseases were investigated in Syrian golden hamsters observed for life in the Eppley and Hannover (Germany) colonies. The majority of neoplasms were detected microscopically by graded sectioning. Of 500 EC (Eppley Colony) hamsters, 20.6% presented tumors, while in the HC (Hannover Colony), 22.3% of 377 hamsters had spontaneous neoplasms. Multiple primary lesions occurred in 42.7% and 29.8% of the EC and HC animals, respectively. Malignant tumors totalled 14.6¢ in the EC and 31.0% in the HC. Cumulative percentages of tumor-bearing animals in terms of sex and tumor types varied significantly between the two colonies during the course of the study. As a result of this study and because of other reports of spontaneous neoplasms, it is mandatory that control groups and histological examinations of control animals be provided in all carcinogenesis studies.

2. Research on the Mechanism of Carcinogenesis -- Basic research in this segment of the program is conducted at the chemical and host levels to determine the biological mechanisms of the carcinogenic process and to define the factors which may control or alter the course of neoplastic growth. A variety of research approaches are employed. The molecular aspects of carcinogenesis, for example, are actively investigated through in vitro studies of both normal and chemically-transformed cells. Also, the anatomical and metabolic fate of chemical carcinogens is examined, and sites of elective localization in terms of tumorigenesis, relative to the various compounds, sought. Possible routes of detoxification and excretion are also explored. Identification of each carcinogen's most active form, characterization of hormonal or genetic host factors critical to carcinogenic responses, and response comparisons in different species are the other integral parts of this program.

Highlights of selected projects under research on the mechanism of carcinogenesis are as follows: (a) Isopropylvaleramide (IVA) and allylisopropylacetamide (AIA) are powerful inhibitors of the acute toxicity of 7,12-dimethylbenz[a]-anthracene (DMBA) in rats. The minimal, but fully protective, dose of IVA is 43.6 μmoles/kg. On the other hand, these two amides have no influence on the acute toxicity of dimethylnitrosamine and increase hepatic damage and the consequent incidence of mortality due to aflatoxin B_1. Since the three carcinogens mentioned are believed to require metabolic activation by microsomal mixed-function oxidases, the differential effect of IVA and AIA on their toxicity is remarkable. IVA has no detectable influence either on the in vivo or in vitro metabolism of DMBA. Yet, at least at higher dose levels, it prolongs hexobarbital sleeping time and paralysis induced by zoxazolamine. The anti-DMBA effect appears to be quite specific for IVA and AIA since related compounds such as formamide, acetamide, butyramide, isovaleramide and 2-ethyl-butyramide proved ineffective in counteracting DMBA-induced toxicity.

(b) Cytochrome P450, the terminal oxidase and the substrate-binding site for the mixed-function oxidase system, contains Fe(II) and Fe(III) chelate complexes. This enzyme in the oxidized form on complexation with an aromatic hydrocarbon might accept one electron directly from the hydrocarbon, a strong donor, to form a reduced substrate-cytochrome P450 complex. Oxidation of the substrate would generate a reactive intermediate radical cation capable of binding to cellular nucleophiles. Radical cations of benz[a]anthracene (BA), 7-methyl-benz[a]anthracene (7,MBA), DMBA and 3-methyl-cholanthrene (MC) were generated by iodine-induced one-electron oxidation and trapped with pyridine. BA, 7-MBA and MC gave a single pyridinium salt, namely, 7-N-pyridinium-BA, 7-N-pyridinium-methyl-BA and 1-N-pyridinium-MC, respectively, while DMBA afforded 7-N-pyridiniummethyl-12-MBA, 12-N-pyridiniummethyl-7-MBA and 5-N-pyridinium-DMBA. The reactivity of the radical cations toward nucleophilic trapping is conferred by a significant degree of positive charge localization, which would account for their covalent binding to cellular nucleophiles and presumably for their carcinogenicity. (c) The possible implication of the 1-carbon atom in the in vivo binding reaction of 3-methylcholanthrene (MC) was determined by measuring the relative carcinogenicity of the hydrocarbon with that of MC having the three hydrogens at the 1- and 5-carbon atoms replaced by deuterium ($MC\text{-}d_3$). If the C-H cleavage at 1-position is the rate-determining step, the

resulting isotope effect could be reflected in a modified carcinogenicity of the deuterated MC. The comparative test between MC and MC-d_3 by repeated skin painting on two groups of 100 female Swiss mice showed a significantly lower tumorigenic activity of the latter as evaluated by statistical analysis. The result implies that the 1-carbon atom of the hydrocarbon is a critical binding-site to cellular targets in the tumor-initiating process and one-electron oxidation of the hydrocarbon could represent an elective mechanism of activation. (d) The study of possible species differences in the metabolism of DDT was continued in an attempt to explain the hepatocarcinogenicity of DDT and its metabolite, DDE, in the mouse, in contrast to the insensitivity of the hamster to this effect. A further conjugate of DDA with serine was identified in both mouse and hamster urine. DDT and DDE pretreatment induced hepatic microsomal drug-metabolizing enzyme activity and cytochrome P450 content in mice. However, DDD, which is not a hepatocarcinogen in mice, had no effect. These effects are being studied with related compounds, and the tumorigenicity of DDT, DDD and DDE is bieng investigated in rats and hamsters. (e) It was proposed that ascorbic acid should be incorporated with readily nitrosable drugs to inhibit their in vivo nitrosation. The induction of lung adenomas in strain A mice was used to test this proposal. In keeping with earlier findings, it was found that ascorbic acid (administered in the diet) resulted in an 89-98% inhibition of lung adenoma induction by nitrite given with piperazine, morpholine, or methylurea. However, ascorbic acid produced 15-59% increases in adenoma induction by two preformed nitrosamines. (f) Thymidylate synthetase (a possible rate-limiting enzyme for DNA biosynthesis) from control and transformed rat embryo cells was also studied. The enzyme's K_m for dUMP was 4.5 μM from both normal and transformed cells. Titration of the enzyme with (^{3}H) FdUMP indicated that the increased enzyme activity in the transformants was due to a greater level of cellular enzyme. The level of enzyme activity in control cells was dependent on the availability of medium nutrients and cell density, while the enzyme level in the transformants depended only on medium nutrients. (g) The gangliosides of control rat embryo cells and their 3-methylcholanthrene and Rauscher leukemia virus transformants and of control mouse embryo cells and their MCA and 7,12-dimethylbenzanthracene transformants were examined using (^{14}C) glucosamine as a tracer. All cell lines exhibited complex ganglioside patterns, however, the transformants showed an increase in the simpler ganglioside, N-acetylneuraminyl-galactosyl-glycosyl-ceramide (GM_3) compared to the respective control cells. (h) Intratracheal instillation of 7-H-dibenz[c,g]carbazole (DBC) and dibenz[a,i]pyrene (DBP) without ferric oxide produced high incidences of lung tumors in Syrian golden hamsters. Benzo[a]pyrene (BP) instilled intratracheally alone has been shown not to be carcinogenic. Lung retention rate studies indicate that DBC is removed at a higher rate (t1/2 = 2.1 hours) than BP (t1/2 = 10.8 hours) and much higher rate than DBP (t1/2 > 24 hours). Particle size analyses indicate similar size distributions for the 3 compounds. The rate of passage of carcinogen through the tissue does not appear to be important in this cancer-inducing system. (i) Some nitrosamines are strong inducers of esophageal tumors in rats, while others are not. The inhibition of ^{3}H-thymidine incorporation into DNA or rat esophageal epithelium was studied 4 hours after i.p. injection of nitrosamine. The dose (in mg/kg) producing 30% inhibition was 1.2 for methylamylnitrosamine, 3.8 for nitrosopiperidine (both strong esophageal carcinogens), and 13 for dimethylnitrosamine and 20 for nitrosopyrrolidine (both esophageal

noncarcinogens). Thus, a significant correlation may exist between this effect and esophageal carcinogenicity. This could be useful as a rapid in vivo prescreen and in understanding the mechanism of organ-specific action. (j) Substituted phenols are major components of hardwood smokes, which are applied to many nitrite-cured foods. Reaction of phenols with nitrite to form nitrosophenols and other products could compete with nitrosation of amines to inhibit N-nitroso compound formation. In model reaction mixtures, 2,6-dimethoxyphenol (syringol), the major phenolic component of wood smoke, provided 92% blockage of nitrosomorpholine production from nitrite and morpholine at pH3. The extent of blockage was equivalent to that produced by an equivalent concentration of ascorbic acid. Some other phenolic components of wood smoke which blocked nitrosomorpholine formation were phenol (15%), guaiacol (71%) and 4-methylguaiacol (81%). (k) Methods for sampling, concentration and detection of trace levels of volatile carcinogens in air are under development. Metal tubes, packed with a porous polymer (Tenax-GC), concentrate the components of interest while minimizing interference from water vapor. Use of these tubes permits remote sampling and storage of collected samples prior to analysis. Fluorocarbon permeation tubes are employed for production of standard atmospheres contaminated with low levels of nitrosamines. Gas chromatography, employing the Hall electrolytic conductivity detector in the nitrogen mode, is used for detection and quantitative measurements with confirmation of identity by combined gas chromatography-mass spectrometry. At the current stage of development, dimethylnitrosamine and diethylnitrosamine can be measured readily at the one part per billion level in air. This method will be applied to monitoring of laboratory and animal room atmosphere.

Significance to Biomedical Research and the Program of the Institute: The Eppley Institute has provided over the years a considerable output of major research results in chemical carcinogenesis and represents a major national resource in which chemical, pathological, and biological aspects of carcinogenesis are studied in an integrated program.

Proposed Course: This contract will continue to support the study, improvement and conduct of bioassay techniques in carcinogenesis. The project will continue as outlined in the objectives above.

Date Contract Initiated: March 19, 1968

Current Annual Level: $2,993,753

THE SALK INSTITUTE (NO1-CP-33405)

Title: Carcinogenesis and Control of the Cell Cycle

Contractor's Project Director: Dr. Robert W. Holley

Project Officer (NCI): Dr. Andrew Peacock

Objectives: The growth of normal and tumor cells is controlled in part by the genome of the cell and in part by extracellular agents. In addition to the

supply of common nutrients (e.g. amino acids, sugars, etc.) the extracellular fluid contains hormones and analogous control substances, some of which influence the availability of the nutrients to the cell. Carcinogenesis changes the relationship between the cell and the extracellular agents such that growth rates greater than normal occur. However, it has been found that some regulations of growth still remain in chemically-induced tumor cells and the residual controls in these malignant cells is the subject of the present study. The overall objective of this study is to determine the kinds of changes that accompany chemical transformation of cells. The present project contributes to that goal, but focuses on mechanisms whereby the growth rate in chemically-induced tumors might be checked.

Major Findings: Major efforts during the last year have been directed toward understanding the changes in growth control that take place when 3T3 cells are transformed by benzpyrene. The work has been carried out with two clones of benzpyrene-transformed 3T3 cells obtained from Dr. Joseph DiPaolo (NCI). Major findings can be summarized as follows.

Comparison of the growth curves, and the final cell densities, of the cells grown in medium with various concentrations of serum reveals no difference in growth behavior between the benzpyrene-transformed (BP3T3) cells and viral transformed (SV3T3) cells. Both transformed cell lines grow to a very high cell density in medium with 10% serum and both have low serum requirements compared with normal 3T3 cells.

Examination of the growth behavior of the two transformed cell lines in detail, however, shows there are several major differences.

BP3T3 cells require a serum factor that is not required by SV3T3 cells. This serum factor appears to be the fibroblast growth factor (FGF) that is required by 3T3 cells.

In contrast to SV3T3 cells, which continue to traverse the cell cycle slowly in low serum, BP3T3 cells arrest in G_1 (or G_0) when growth is limited by serum. Quiescent BP3T3 cells obtained in this manner initiate DNA synthesis in response to FGF. FGF is not required by SV3T3 cells. For continued growth of BP3T3 cells with FGF in low serum, a high molecular weight factor from serum, or brain extract, must also be added; this high molecular weight factor appears to be the same factor that is required for rapid growth of SV3T3 cells.

BP3T3 cells also arrest in G_1 (or G_0) when the cells reach the plateau phase after growth in medium with 10% serum. Under these conditions growth is arrested by exhaustion of the amino acids in the medium. In contrast, SV3T3 cells do not arrest in G_1 (or G_0) when they reach plateau phase in 10% serum.

In summary, the benzpyrene-transformed cells retain growth controls similar to those observed in normal 3T3 cells, but with partial relaxation of these controls. Apparently this is sufficient to make the cells tumorigenic. Since "minimal-deviation" tumors can provide insight into the minimal changes necessary for carcinogenesis, it will be of interest to determine the mechanism of relaxation of the controls. Techniques are now available for such studies.

Significance to Biomedical Research and the Program of the Institute: This contract will attempt to provide more detailed information regarding the relationship between extracellular factors, membrane controls and intracellular factors in normal and chemically-transformed cells. This can provide insight into the rates of growth of different types of cancers which can serve as a basis of considering new means of controlling malignant growth.

Proposed Course: Growth control mechanisms will be studied in various chemically-induced tumor cells and in cells transformed chemically in vitro. From past experience, it is anticipated that some of these controls will involve previously unrecognized components of serum and attempts will be made to identify these components. Attempts to identify other growth regulatory mechanisms in chemically-transformed cells will involve manipulation of the composition of the medium as well as manipulation of serum fractions. There will be a search for interactions between growth regulatory mechanisms. An attempt will be made to understand the fundamental changes that lead to changes in growth control in chemically-transformed cells.

Date Contract Initiated: June 26, 1972

Current Annual Level: $152.472

SUMMARY REPORT

BIOASSAY OPERATIONS SEGMENT

July 1, 1974 through June 30, 1975

Plan of Operation and Program Activities

The primary goal of the Bioassay Operations Segment is the identification and evaluation of chemical carcinogens, particularly those of environmental and occupational significance. This goal is pursued through the bioassay of these chemicals, either singly or in combination, in long-term animal studies. At the same time, the Bioassay Operations Segment is attempting to improve the sensitivity and reproducibility of present bioassay systems as well as to develop new ones.

After a chemical is selected for bioassay (described below) a number of sequential activities are undertaken. These include (1) evaluation of all known safety data and other relevant information to determine testing priority; (2) procurement, chemical analysis, and work-up of special procedures; (3) assignment to a bioassay laboratory; (4) pre-chronic toxicity testing; (5) long-term bioassay and pathology evaluation of its effects; (6) analysis and evaluation of the data; and (7) preparation of a Technical Report. A large amount of advance planning is necessary to ensure the successful and efficient accomplishment of each stage. Resources are needed to provide analytical capability, animals, and data management. Coordination of these resources are needed to ensure that high quality animals of a specified age are available at the proper time and in sufficient quantities, that the bioassay laboratory has adequate manpower to effectively conduct each phase of the study, and that the data are collected and made available in a timely manner. During the performance of the bioassay the progress and results are continuously monitored. If the results indicate that the chemical may be a potential human health hazard, the Department of Health, Education and Welfare and the relevant regulatory agencies are notified.

The actual bioassay consists of a pre-chronic and a chronic phase. During the former, the maximum tolerated dose of the chemical that can be given in the chronic phase is predicted. The chronic phase must be carefully planned to assure that the proper experimental design is used and that the bioassay is conducted under optimal conditions. Particular attention must be given to ensure the safe handling and disposal of the test chemical and waste materials. Data are provided to the Carcinogenesis Bioassay Data System (CBDS) on the experimental design, clinical and survival observations, and pathology diagnoses. Analysis of the data can be made at any time during the study. After all the data have been appropriately analyzed and evaluated, they are published in scientific journals and/or as part of a comprehensive Technical Report.

Because of the insufficient staff manpower available for the management of the bioassay program it was decided to establish a prime contract which

would provide the necessary management effort under the scientific direction of this Segment's staff.

After an extensive competitive evaluation, the Bioassay Prime Contract was awarded in March, 1974 to Tracor Jitco, Inc. (NO1-CP-43350). The Tracor Jitco staff has relieved the members of the Bioassay Program of much of their immediate contract-related administrative and management duties. As a result, the NCI staff has had more time to concentrate on the scientific needs of the Program.

Although the Bioassay Prime Contractor has direct control over many of the bioassay laboratories, the NCI still retains ultimate responsibility for all of the Program's activities. The Bioassay Program staff members are kept aware of the activities of the Bioassay Prime Contractor through daily communication, reports, and frequent progress meetings.

At the start of the fiscal year the Bioassay Operations Segment was directly responsible for twelve contracts, one of which has terminated. The Bioassay Prime Contractor had an additional four projects that were acquired as subcontracts in FY 1974. During the current fiscal year the Bioassay Prime Contractor has acquired another six subcontracts which have been transferred from direct NCI management. Thus, the transition of these efforts has resulted in the Bioassay Prime Contractor becoming responsible for a total of ten subcontracts and the Bioassay Operations Segment retaining control of five contracts.

The Bioassay Operations Segment also provides support for and monitors the large-scale bioassay project at the NCI Frederick Cancer Research Center (NO1-CO-25423). During the fiscal year the Segment provided partial funding for pathology support and for data management contracts, both of which are administered by the Information and Resources Segment.

During the fiscal year approximately 540 chemicals were in one or another stage of the testing program. Of these 112 were pharmaceutical products, 82 industrial chemicals, 94 pesticides and agricultural chemicals, 36 metallic compounds, 31 natural plant products, and 9 food additives. Most of the remainder have multiple uses or are structural analogs tested for structure-activity relationships. Approximately 225,000 animals have been used during the fiscal year. These include about 105,000 rats of the Osborne-Mendel, Fischer/344, and Sprague-Dawley strains; 105,000 mice of the Swiss, C57BL/6, and hybrid B6C3F1 strains; and 15,000 hamsters and other species.

Figures 1 through 4 illustrate the organization of the Bioassay Operations Segment including Prime Contract, supporting or resource activities, various cost estimates, and time requirements for each phase.

Chemical Selection Process

The bioassay of each chemical for carcinogenicity requires a substantial commitment in time and money. Three years or longer may be needed to complete each bioassay at a cost of $100,000 or more. Thus, every effort must be made to ensure that each chemical nominated for bioassay is thoroughly

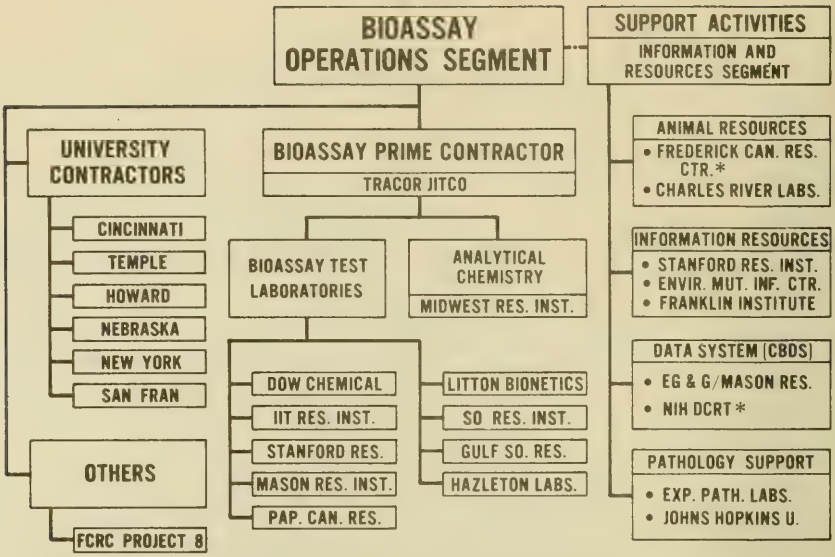

Figure 1. Organization of Bioassay Operations Segment

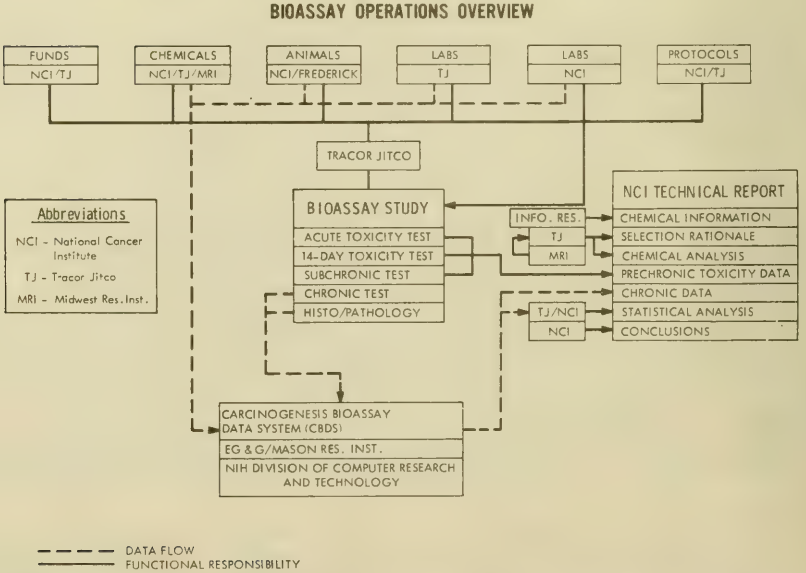

Figure 2. Review of Bioassay Operations Indicating Responsibilities and Flow of Research Data to CBDS and Technical Report Preparation

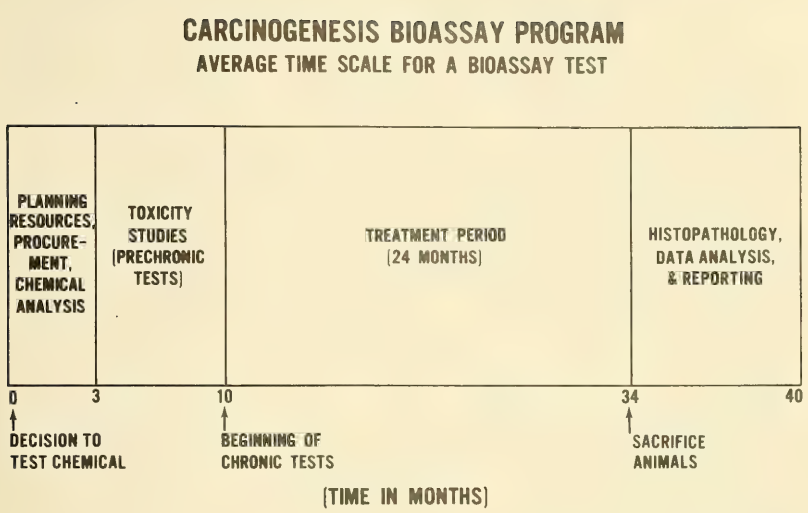

Figure 3. Average Time Requirements to Complete Each Phase of a Carcinogenesis Bioassay Test

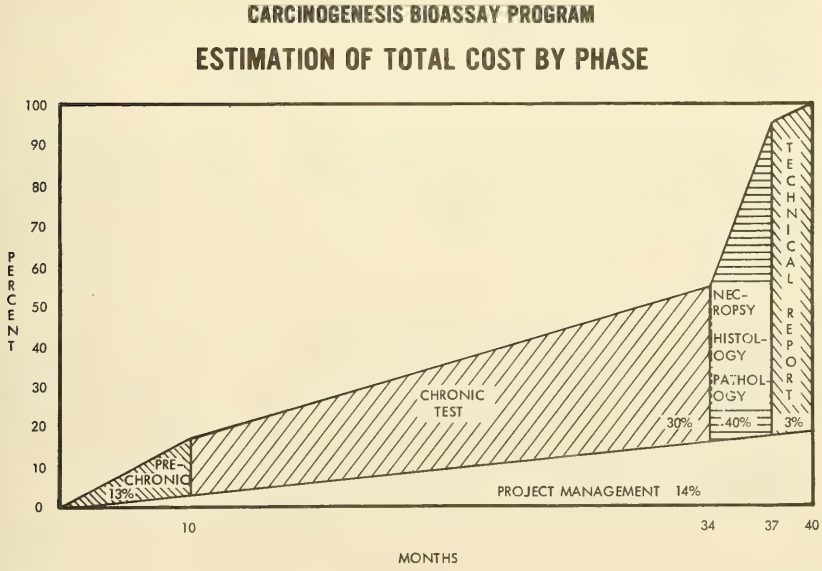

Figure 4. Average Percentage of Total Cost for Various Phases Involved in a Carcinogenesis Bioassay Test

evaluated before resources are committed to it. A Chemical Selection Working Group (CSWG) has been established to carry out this critical evaluation. The CSWG is chaired jointly by Dr. Elizabeth K. Weisburger, Chief, Carcinogen Metabolism & Toxicology Branch, NCI and Dr. Carl Wessel, Bioassay Prime Contract Project Director. Nomination of chemicals for consideration by the CSWG are received from NCI staff, from other Federal agencies, from information contracts, and from the scientific and technical community. For each chemical, the CSWG endeavors to gather as much information as is practically possible, including a summary of the experimental studies reported in the open literature regarding its toxicity, information on its occurrence, production, use, and human exposure, as well as data on its physical and chemical properties, structural relationship to known carcinogens, metabolism, and epidemiological observations. A summary of this dossier is prepared along with the CSWG's recommendation and submitted to a Chemical Selection Committee (CSC). The CSC has the ultimate responsibility of determining which chemicals are selected for bioassay. The CSC is expected to initiate its activity by the end of this fiscal year: its members will be from NCI staff and the chairman will be Dr. Umberto Saffiotti; Associate Director for Carcinogenesis, DCCP; ad hoc outside expert consultants will be used. In the eight months that the CSWG has been in existence, approximately 1000 chemicals have been considered, of which about 90 have been recommended for bioassay.

Experimental Design

A major activity during this past year has been the review of existing carcinogenesis testing methodologies and the preparation of detailed NCI Guidelines for Carcinogen Bioassay in Small Rodents. These Guidelines, described in the Carcinogen Bioassay & Program Resources Branch report, have been implemented in routine carcinogenesis tests conducted by the Bioassay Operations Segment.

Bioassay Reports and Data Analysis

One of the most important aspects of the Bioassay Program is the collection, analysis, and reporting of data. The data from studies initiated within the last two and half years have been entered into the computerized Carcinogenesis Bioassay Data System (CBDS). Data from earlier studies have been collected and stored in progress reports and other formal documents. The CBDS collects, monitors, and stores bioassay data. The system has been designed for the complete or selective recall of these data. In addition, the CBDS contains chemical and bioassay sub-systems that provide information on each chemical under test or projected for study as well as other data related to them. During the year, emphasis was placed on refining the data input to CBDS and developing a capability for the routine generation of data tables. The data entry, SNOP-coding, and similar functions are done under contract to Wolf Research & Development Corporation (NO1-CP-43257) (administered and partially funded by the Information and Resources Segment).

The need for a competent and thorough evaluation of the bioassay data is self-evident. However, since neither the Bioassay Program nor many of the

contractors have the capability to analyze data, the assistance of biostatisticians from outside of the Program has been sought. The primary source of help has come from the statisticians associated with the Field Studies and Statistics Program of DCCP.

Although bioassay contractors are encouraged to submit their testing results to scientific journals, it is necessary that the data be properly tabulated and evaluated prior to their publication. For this reason, all bioassay data must be reviewed and approved by the NCI (Bioassay Program) prior to their release. However, because of their voluminous nature, publication of all the data collected from a bioassay study is not possible. Therefore, the scientific community rarely has the opportunity to review the bioassay data in their entirety. As a result, the preparation of a Technical Report Series has been undertaken to provide complete documentation of each bioassay study. A technical Report will be prepared on each chemical tested and will contain complete information, from the rationale for its bioassay through an interpretation of the test data.

Bioassay Resources

The major resources needed to conduct a carcinogen bioassay study are (1) analytical chemistry, (2) animal production, (3) testing laboratories, and (4) data management. The Bioassay Program's analytical chemistry resource is located at the Midwest Research Institute. Midwest Research Institute, a subcontract to the Bioassay Prime Contractor, is responsible for characterizing the test chemicals as well as determining their proper mixing and handling procedures. The NCI Frederick Cancer Research Center (NO1-CO-25423) is the primary source of animals used in the Bioassay Program. The mice and rat colonies at FCRC are periodically restarted with breeders obtained from the NIH Division of Research Services. The testing laboratories must meet certain minimum standards before they qualify to participate in the Bioassay Program. Foremost among them are their ability to safely handle the test agents and provide the needed capability to ensure long-term animal survival. The Carcinogenesis Bioassay Data System (CBDS) is the Bioassay Program's main data resource. The CBDS is operated under contract (administered and partially supported by the Information and Resources Segment) by the Wolf Research & Development Corporation (NO1-CP-43257). The main function of the CBDS is to collect, selectively retrieve, and report data collected in the Bioassay Program. Besides these major resources, close collaboration has been developed and maintained with other members of the Carcinogenesis Program's staff.

The increase in number of chemicals placed on test in the FY 72-73 period is now realized by large workloads in pathology evaluation, data analysis, and preparation of reports. As the Technical Report series is still in the developmental stage, contractor scientists have continued to report their test results by the usual methods, i.e., scientific journals, conferences, progress reports, etc. A list of chemicals reported to have carcinogenic activity in the animal bioassay tests is presented as Table 1.

Table I

CHEMICALS REPORTED TO HAVE SHOWN CARCINOGENIC ACTIVITY
BY INVESTIGATORS SUPPORTED BY THE CARCINOGENSIS PROGRAM
IN FISCAL YEAR 1975

NOTE: In some cases the data have not been completely reviewed by the NCI staff and the designation of carcinogenic activity by the investigator does not necessarily represent an official NCI position. In addition, carcinogenesis effects were reported by other chemicals already well established as carcinogens.

1-Acetyl-2-isonicotinoyl-hydrazine
1-Acetyl-2-picolinolhydrazine
Acronycine
Actinomycin D
Aldrin
2-Aminoanthraquinone
3-Amino-4-ethoxyacetamide
3-Amino-9-ethylearbazole
1-Amino-2-methylanthraquinone
Amitrole
n-Amyl hydrazine HCl
Azaserine
Butane sultone
n-Butyl hydrazine HCl
N,N-bis(2-chloroethyl)-N'-3-hydroxypropyl-phosphoro-diamidic acid
Carbamylhydrazine HCl
1-Carbamyl-2-phenyl-hydrazine
Chlorambucil
Chlordane
Bis-1,2-(chloromethoxy)ethane
Bis-1,4-(chloromethoxy)-p-xylene
1-Chloro-4-nitrobenzene
1-Chloro-2-nitrobenzene
4-Chloro-o-toluidine HCl
p-cresidine
Cupferron
Cytoxan
Dapsone
DDE
DDT
Diazoacetic ester
7-H-dibenzo[c,g]carbazole
Dibenzo[a,i]pyrene
1,2-Dibromo-3-chloropropane
Dibromodulcital
Dibromomannitol
1,4-Dichlorobutene-2
2,3-Dichloro-p-dioxane
Dieldrin

Diethyl-β-γ-epoxy-propyl phosphonate
4,4'-Bis(diethylamino) benzophenone
2,2'-Dihyroxy-di-n-propylnitrosamine
2,5-Dimethoxy-4'-aminostilbene
Dimethylcarbamyl chloride
1,2-Dimethylhydrazine
4(5)-(3,3-Dimethyl-1-triazeno)imidazole-5(4)-carboxamide
Dioxane
Di-n-propyl-nitrosamine
Dulcin
Epichlorohydrin
Ethyl bromoacetate
Ethylene dibromide
Ethylhydrazine HCl
Ethylene thiourea
Extracts from various plants (mostly tannin-containing fractions)
Heptachlor
2-Hydroxypropyl-propyl-n-nitrosamine
Isophosphamide
Melphalan
Metals (certain metals and derivatives, by intramuscular route)
Methyl hydrazine
Methyl-propylnitrosamine
4,4'-Methylene-bis-(2-chloroaniline HCl)
Mirex
Mitomycin C
Niridazole
Nitrilotriacetic acid
5-Nitroacenaphthlene
5-Nitro-o-anisidine
4-Nitroanthranilic acid
2-Oxopropyl-n-propylnitrosamine
o-Phenylene diamine 2HCl
Procarbazine (Natulan)
Propane sultone
Propyleneimine HCl
Sodium azide
Tetrafluro-m-phenylene diamine 2HCl
Thio-TEPA
o-Toluidine HCl
2,4,6-Trichloroaniline
Trichloroethylene
2,4,5-Trimethylaniline HCl
2,4,6-Trimethylaniline HCl
Tris-1,2,3(chloromethoxy)propane
Uracil mustard
2,5-Xylidine HCl

Major Findings

Some major findings obtained by projects of the Bioassay Operations Segment during the fiscal year are described below.

1. Projects supported by contracts directly managed by the Segment.

The native population of the Island of Curacao in the Caribbean exhibits an unusually high incidence of esophageal cancers. It has been suggested that these may be related to the use of herbaceous folk remedies. At Howard University (NO1-CP-33266) a number of these herbs are being fractionated and screened for carcinogenicity by subcutaneous administration to rats. For those herbs that have been found to contain carcinogenic materials, the activity was mainly associated with the tannin-containing fractions.

A variety of alkylating agents used in the chemical industry were tested for carcinogenicity at the New York University Medical Center (NO1-CP-43221). Several of the agents were found to be carcinogenic in mice. Studies have been conducted by various routes of administration and demonstrate that the route of exposure can often influence the outcome of the test.

A project at Temple University (NO1-CP-43271) has established experimental methods for investigating the effects of changes in the "effective thickness" of stratospheric ozone on UV photo carcinogenesis.

2. Projects conducted by subcontractors to Tracor Jitco, Inc. (NO1-CP-43350).

At Gulf South Research Institute twenty pesticides are being tested for carcinogenic activity. An important finding has been that for many of the organochlorine compounds an initial dose level of about 25 percent of that which appeared to be the maximum tolerated level, based on six-week pre-chronic studies, would have more closely approximated the true MTD for the length of the chronic phase. Several of these pesticides appear to be carcinogenic and are now undergoing detailed evaluation.

A total of forty-three environmental chemicals are under investigation for carcinogenicity at Hazleton Laboratories. Inhalation studies have been initiated on two halogenated aliphatic compounds (ethylene dibromide and dibromochloropropane) which were reported last year to be highly carcinogenic when given orally to both mice and rats. For a number of other chemicals, evidence of carcinogenicity is developing; these are now in different stages of evaluation.

Carcinogenicity studies are underway on a number of dibenzodioxins at the IIT Research Institute. Many of these highly stable environmental contaminants are extremely toxic and tend to accumulate in the food chain. Pre-chronic toxicology has been completed for tetra- and hexa- chlorodibenzodioxins and their chronic study is now being planned.

Forty-two chemicals are under test at Litton-Bionetics; nineteen of them have completed the treatment period in mice and twelve of them in rats. The necropsy findings indicate that several of the compounds appear to be

carcinogenic in one or both species; the tissues from these animals are now being evaluated.

At the Southern Research Institute a number of chemotherapeutic drugs and related chemicals are being tested for carcinogenicity. Each chemical is administered by a route comparable to human exposure. Procarbazine induced tumors of the nervous system in both mice and rats. A high incidence of reticulum cell sarcoma was found in mice treated with isophosphamide. Other chemicals that have been tested have also demonstrated varying degrees of carcinogenic potential.

A comparative study of six inbred strains of rats is being concluded at the Papanicolaou Cancer Research Institute. These strains include the Fischer line 344, A x C line 9935 Irish, August line 990, Marshall line 520, S x F line 40814, and Zimmerman line 61. With the carcinogen N-OH-N-2-fluorenyl-acetamide, the most frequent site of tumors was in the liver. The next most frequent tumor type was squamous cell carcinomas of the stomach. About 35 percent of these tumors were found in the August line 990 rats. Nearly half of the control rats are still alive.

CINCINNATI, UNIVERSITY OF (NO1-CP-33202)

Title: Study of the Carcinogenic and Co-carcinogenic Properties of Industrial Chemicals

Contractor's Project Director: Dr. Eula Bingham

Project Officer (NCI): Dr. Henry Hennings

Objectives: (1) To bioassay mixtures of pure compounds which are of occupational and environmental interest to determine the modifying effects of certain physical agents (UVL) and non-carcinogenic chemicals on carcinogenic potency of known carcinogens and (2) to determine factors contributing to the carcinogenic potency (skin and lung) of complex mixtures derived from combusting fossil fuels.

Major Findings: It was demonstrated that n-paraffins enhance the rate of appearance and number of tumors in mice exposed to UVL at 254nm and 290-320nm. In addition, using a "non-carcinogenic" wavelength (black light) >350nm, the mice receiving topical applications of n-dodecane and n-tetradecane developed tumors.

Several methyl derivatives of benzanthracene which are considered non-carcinogenic or weakly carcinogenic were demonstrated to be moderate to strong carcinogens when applied using n-dodecane as the solvent.

Significance to Biomedical Research and the Program of the Institute: Relevance of experiments to determine the factors modifying the potency of carcinogens, weak carcinogens, and non-carcinogens found in fossil fuels appears to be obvious with the increased conversion and utilization of sources such as coal and shale. Both the skin and lungs are target organs in exposures from these agents. Other organs such as the kidney have been shown to be at risk from exposure to emissions derived from the coking of coal. A model for the development of lung cancer by complex mixtures using inhalation techniques would be a great asset in chemical carcinogenesis.

It is important that adequate recognition be made of modifying agents (carcinogen and inhibitors) in any assessment of risk to human populations and prevention of disease.

Proposed Course: The contract will terminate on November 30, 1975. It is proposed to bring ongoing work to an orderly and logical conclusion.

Date Contract Initiated: September 11, 1963

Current Annual Level: $72,588

HOWARD UNIVERSITY (NO1-CP-33266)

Title: Chemical and Biological Investigation of Potential Carcinogens from Plants

Contractor's Project Director: Dr. Govind J. Kapadia

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: (1) To prepare purified fractions from certain herbs that are used habitually in Curacao and South Carolina and isolate and characterize crystalline compounds from these fractions, (2) to test each fraction and compound for carcinogenicity in experimental animals and identify substances responsible for the carcinogenic effects, (3) to extend such chemical and bioassay studies to plant species related to the above as well as to other plants that are suspected of possessing carcinogenic activity, and (4) to elucidate the chemical structure of any carcinogenic substance that may be isolated.

Major Findings: This project is a continuation of earlier investigation which was developed in an attempt to correlate the high incidence of esophageal carcinoma in natives of certain places with their habit of using herbaceous folk remedies. Thus far 61 plant samples (belonging to 52 genera and 57 species, procured from Curacao, South Carolina and other places) have been extracted with water; the aqueous extracts have been lyophylized and submitted for bioassay. The aqueous extracts of tannin-bearing plants were fractionated to yield tannin and tannin-free fractions which were also provided for bioassay.

For bioassaying these materials, subcutaneous injections in suitable doses were given to 15 male and 15 female NIH black rats once a week up to 72 weeks. The observation period ran up to 90 weeks. The extracts and fractions that produced tumor and the number of rats developing tumor to the total number used to date are as follows: Krameria ixina aqueous extract (54/90), tannin-free fraction (3/60), and tannin (28/30); K. triandra aqueous extract (30/30), and tannin (28/30); Sorghum vulgare (bird resistant variety), aqueous extract (0/30); Acacia villosa aqueous extract (30/30), and tannin (29/30). Among these plants, A. villosa appeared to be the most potent carcinogen.

In subsequent studies a total of 16 extracts and fractions from 8 other plants were used. The results of these bioassays were: Camellia Sinensis (Assam tea), aqueous extract (6/30), tannin (26/30); Myrica cerifera aqueous extract (9/30), tannin (13/30), tannin-free fraction (15/30); Limonium nashii aqueous extract (18/30), tannin (10/30); Quercus fulcata pagodaefolia, aqueous extract (30/30), tannin (28/30); Liquidambar styraciflua, aqueous

extract (17/30), tannin (24/30); Diospyros virginiana, aqueous extract (0/30), tannin (19/30). Histologically, the induced tumor showed characteristics of malignant fibrous histiocytoma, that manifested low grade malignancy, but usually multi-nodular growth pattern.

The most significant part of the results is that when tea (Camellia sinensis), which is commonly consumed as a beverage, was administered subcutaneously the rats developed tumors. This plant needs to be further investigated for carcinogenicity.

Significance to Biomedical Research and the Program of the Institute: This project is directly related to the objectives of the Carcinogenesis Program, i.e., the identification of unknown carcinogens in natural plant materials. This project should serve as the ultimate check to ascertain the carcinogenicity of materials implicated by epidemiological studies on the island of Curacao. Information gained from this study could conceivably lead to definitive studies that should be conducted in other high incidence areas to identify the carcinogens involved.

Proposed Course: It is proposed to isolate and characterize the chemical compounds in plant extracts found to be carcinogenic. Furthermore, additional plants will be biologically and chemically investigated.

Date Contract Initiated: June 25, 1971

Current Annual Level: $130,144

NCI, FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) (NO1-CO-25423)

Project #8

Title: Bioassay Research

Contractor's Project Manager: Dr. Borge Ulland

Project Monitor (NCI): Dr. Norbert Page

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

NEW YORK UNIVERSITY (NO1-CP-43221)

Title: Alkylating Agents as Carcinogens

Contractor's Project Director: Dr. Benjamin L. Van Duuren

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: A wide variety of alkylating agents are being introduced into the environment. Some of these compounds are encountered in manufacturing processes where they may be occupational hazards. Others become distributed in the environment to greater or lesser extents. The purpose of the work carried out on this contract is to determine the possible carcinogenic activity of some of these compounds in laboratory animals using several routes of exposure and to explore chemical structure-activity relationships. A major aim of the work is to obtain biological information that can lead to the reduction or elimination of human exposure to these agents.

Major Findings: During the past year tests have been completed on a series of chloro ethers and related compounds. The methods used were mouse skin application, initiation-promotion experiments, subcutaneous injection in mice and intraperitoneal injection also in mice, for some of these compounds. In addition, the relationship between chemical structure, chemical reactivity, and carcinogenic activity was examined in detail.

Based on the bioassays the following compounds showed significant carcinogenic activity by one or more routes: dimethylcarbamyl chloride, ethyl bromoacetate, epichlorohydrin, 2,3-dichloro-p-dioxane, glycol sulfate, diethyl-β, γ-epoxypropylphosphonate, bis-1,2(chloromethoxy)ethane, bis-1,4-(chloromethoxy)-p-xylene, tris-1,2,3(chloromethoxy) propane and 1,4-dichlorobutene-2. The compounds underlined merit further biological testing since some of them are known to be used in chemical industry (e.g., epichlorohydrin, 1,4-dichlorobutene-2 and dimethylcarbamyl chloride) and the others are either in use or are likely candidates for use in chemical industry.

The chemical reactivity of 27 of the compounds that were tested for carcinogenicity was examined by reaction with various nucleophilic chemicals. These studies showed that several parameters have to be considered in attempting to predict the carcinogenic activity of alkylating agents.

Significance to Biomedical Research and the Program of the Institute: One accomplishment of this program has been to pinpoint several industrial chemicals as occupational carcinogens. Two of these compounds, chloromethyl methyl ether and bis(chloromethyl) ether have, as a result of studies initiated under this contract, been classified as human carcinogens. Several others uncovered in these studies are suspect human carcinogens but require additional bioassays and, where possible, epidemiologic studies. Studies on chemical structure and carcinogenicity relationships carried out under this contract have provided extensive new background information on which to base prediction of carcinogenic activity amongst direct-acting alkylating agents. Finally, the studies carried out under this contract have stimulated a renewed interest in and awareness of the carcinogenic potential of alkylating agents, their mode of action in carcinogenesis, and the serious hazard some of these compounds present as occupational carcinogens.

Proposed Course: Contract terminated October, 1974.

Date Contract Initiated: October 1, 1970

Current Annual Level: $70,708 (for eight months)

SAN FRANCISCO, UNIVERSITY OF (NO1-CP-33229)

Title: Study of the Carcinogenicity of Heavy Metals

Contractor's Project Director: Dr. Arthur Furst

Project Officer (NCI): Dr. John A. Cooper II

Objectives: To determine the potential carcinogenicity of selected metals of industrial or environmental significance.

Major Findings: Under the test conditions, very few of the metals studied were found to be carcinogenic in the Fischer strain rat. Only nickle and cadmium were found to be definitely positive, producing fibrosarcomas at the injection site in six to ten months. Chromium metal and manganese acetonyl-acetate produced only a few local fibrosarcomas. Other metals such as iron, copper, gold, silver, lead, and zinc failed to produce tumors. The zinc also failed to negate the cadmium carcinogenicity. It appears that nickle and cadmium effects are additive.

Significance to Biomedical Research and the Program of the Institute: An accumulation of a single heavy metal in a vital organ may cause toxic or even carcinogenic effects. Foreign metals may displace essential ones required for enzyme function; this may lead to inhibition of enzyme action and mal-functioning of a vital organ. The heavy metal may also inhibit an enzyme without displacing the essential metal. An example would be the inhibition of aryl hydrocarbon hydroxylase, a vital enzyme in the liver and lungs that detoxifies the carcinogenic hydrocarbons. The heavy metals are not bio-degradable, thus once they get into the eco-system from burning of coal or oil or as waste products of industry they may pose a carcinogenic hazard.

Proposed Course: To conclude ongoing studies, collate the data, and prepare manuscripts for publication.

Date Contract Initiated: June 3, 1964

Current Annual Level: $61,388

TEMPLE UNIVERSITY (SKIN AND CANCER HOSPITAL) (NO1-CP-43271)

Title: UV Photocarcinogenesis: Effects of Depletion of Atmospheric Ozone

Contractor's Project Director: Dr. Paul D. Forbes

Project Officer (NCI): Dr. Norbert Page

Objectives: The primary objective of the proposed investigation is to identify and quantitatively assess the relationship between changes in the "effective thickness" of stratospheric ozone and the development of solar damage to the skin, particularly carcinogenesis. The basic experiment is

designed to give data based on three effective thicknesses of ozone, the principal biologic endpoint being carcinogenesis in the skin of hairless mice. A xenon arc lamp will be used to simulate sunlight, and an optical filtration system will be used to simulate changes in the stratospheric ozone content.

Major Findings: Equipment acquisition and assembly has been completed and initial tests have been conducted. The light source operates according to specifications; gas generation and measurement, light detection and measurement, and safety equipment meet or exceed expectations. The special radiation cage units function well. The computer system is being used to analyze and plot spectral data; programs are being developed for analysis of the biological data.

The ozone optical filter unit has functioned adequately as a part of the electrical-mechanical complex, but full utilization of the system has been delayed by small, persistent gas leaks at the interface between quartz cylinders and teflon-coated end plates. Inert silicon-based sealants appear to be one means of eliminating the problem, and several are being tested. As soon as proper gas flow and containment is achieved in the filter unit the animal exposures phase will begin.

A significant body of data has been generated through the use of the light source, Cary spectroradiometer, Robertson-Berger UV meters, and Wang computer.

Significance to Biomedical Research and the Program of the Institute: This project will yield scientific data relevant to the broad field of cancer biology and in addition provide data of immediate concern to the National Cancer Institute, to the Department of Transportation's Climatic Impact Assessment Program, to NASA, and to other agencies concerned with inadvertent modification of stratospheric ozone. Assessment of the contribution of various wavebands of ultraviolet light to changes in the skin will play an important part in the understanding of naturally-occurring light-induced alterations.

Proposed Course: The ozone filter will be appropriately modified, and animal experiments should be started according to protocol originally outlined.

Date Contract Initiated: February 15, 1974

Current Annual Level: $156,703

TRACOR JITCO, INC. (NO1-CP-43350)

Title: Bioassay Prime Contract

Contractor's Project Director: Dr. Carl J. Wessel

Project Officer (NCI): Dr. Norbert Page

Objectives: To provide administrative and management support to the Bioassay Operations Segment in the conduct of carcinogenesis bioassay testing of environmental chemicals. This entails the following: (1) assume subcontract responsibility for existing NCI contracts as designated by the Bioassay Operations Segment; (2) develop plans, RFPs, advertise and award new competitive subcontracts; (3) develop and implement a management system to coordinate and monitor the research conducted by the subcontractors; (4) propose scientific improvements and cost-saving/efficiency methods for the program; (5) coordinate resources and assure safety in the testing program; (6) provide for data submission to the Carcinogenesis Bioassay Data System and prepare interim and final reports on the chemicals being tested; and (7) make a best effort in assuming other responsibilities as desired.

Major Findings: (1) Ten former NCI bioassay contracts were converted into Tracor Jitco subcontracts. These are Dow Chemical Company, Gulf South Laboratories, Hazleton Laboratories, Illinois Institute of Technology Research Institute, Litton Bionetics, Mason Research Institute, Midwest Research Institute, Papanicolaou Cancer Research Institute, Southern Research Institute, and Stanford Research Institute. (2) Monitorship of the ten subcontracts was continued. (3) A Management Plan and System for the Bioassay Operations was established. (4) A list of qualified laboratories was developed after visits to 27 facilities. (5) A competitive request for proposal (RFP) for initiation of testing of additional chemicals was released and proposals are under review. (6) The nomination, selection, and procurement of chemicals to be tested was accelerated. (7) An information system on chemicals nominated was established. (8) Coordination of animal and chemical resources was assumed. (9) Preparative work on a series of Technical Reports on chemicals tested was commenced. (10) Actions were initiated to improve the data input into the Carcinogenesis Bioassay Data System.

Significance to Biomedical Research and the Program of the Institute: One of the primary factors basic to the understanding and prevention of cancer is the identification of carcinogenic chemicals in the environment of man. If "environment" is defined to include not only the atmosphere but also industry, transportation, clothing, housing, occupation, food, drugs, cosmetics, and any other entity with which man comes into contact, the number of suspect chemicals is very large. Thus, the number of chemicals which must be tested is probably large. Because of the nature of the bioassay, numerous animals must be utilized in testing each chemical over long time periods. Extensive elements of data are produced which must be recorded accurately and interpreted expertly. This convergence of large-dimension factors results in high costs. In order to obtain the greatest value in knowledge with minimal expenditures of funds, it is essential that the program be managed carefully to ensure good science, expert use of manpower and funds, and high quality. A principal goal of the Prime Contrator, therefore, is to achieve the objectives of the NCI Bioassay Operations Segment with high scientific credibility at reasonable costs.

Proposed Course: It is intended to continue all ongoing bioassays of chemicals to completion, with subsequent reporting of results in a series of

Technical Reports. The testing of a substantial number of additional chemicals will be commenced according to a schedule arranged in terms of availability of funds, animals, chemicals, and qualified laboratories. Improvements in the bioassay procedures are planned with respect to sensitivity of animal models to carcinogens and identification of alternative animal models; development of time-dose-response procedures; development of better evaluative procedures which can be used to supplement or replace screening procedures; development of suitable inhalation test protocols; employment of biochemical procedures to give a better understanding of metabolic pathways and detoxication mechanisms involved during carcinogenesis tests; studying the design, environment or host factors that may affect the outcome of bioassay, e.g., feed , waters, biometry, etc., consideration of ways and means to implement cost-savings and efficiency, by improvement in the design of laboratory facilities, streamlined methods of reporting pathology data, etc.

Date Contract Initiated: March 1, 1974

Current Annual Level: $4,261,387

Narrative statements of the ten subcontracts to Tracor-Jitco, Inc. are reported below.

1. Subcontract with Dow Chemical Company

Title: Carcinogenesis Bioassay of Environmental Chemicals

Contractor's Project Director: Dr. J. L. Emerson

Project Officers (Tracor Jitco): Dr. Carl J. Wessel
Dr. Garth Fitzhugh

Objectives: To evaluate the potential carcinogenicity of 5 environmental chemicals; namely, 2-amino-5-nitrothiazole; 1,3-dichloro-5,5-dimethyl-hydantoin; 3 nitropropionic acid; proflavinedihydrochloride; and N,N'-dicyclohexylthiourea. N-2-fluorenylacetamide (2-AAF) is used as a positive control. The studies are conducted using both sexes of F/344 rats and B6C3F1 mice at two dose levels, the MTD and 1/2 MTD groups of 50 animals are used for each dose and sex. The duration of the studies are 110 weeks for rats and 104 weeks for mice. Due to a lack of stability of 3-nitro-propionic acid in the diet it is being administered 5 days per week via gavage. All other chemicals are being administered via the diet which is prepared weekly or bi-weekly. Chronic testing began in March and June of 1973.

Major Findings: The acute oral median lethal dose and subacute 6 weeks toxicity studies were completed for the compounds now under long-term test. In addition, acute and subacute studies on resorcine blue in both species and scopolamine hydrobromide in mice have been completed. Resorcine blue and scopolamine hydrobromide were subsequently dropped from further testing. To date, survival has been good in both rats and mice with the exception of

2-AAF, the positive control. In rats treated with 2-AAF hepatocellular carcinoma with metastasis to the lung has been a constant finding. In addition, interstitial cell tumors of the testes and mononuclear leukemia, as described by Moloney, has been frequently found. With 2-AAF treated mice, bladder carcinoma, hepatocellular carcinoma, or hematopoietic neoplasms have been consistent findings. Leukemia and related tumors are becoming more frequent as the studies progress.

Significance to Biomedical Research and the Program of the Institute: A primary effort in the prevention of cancer consists of determining potential carcinogenic hazards in the environment so that human use and exposure may be minimized. The aim of this program is to gather such data for a number of widely used materials.

Proposed Course: All chemicals are currently in the stage of chronic testing which will continue through July 1975. All animals will be examined at the time of death or at the termination of the study for evidence of neoplasia and chronic toxicity. This is the third year of the contract which is projected to continue through Fiscal Year 1975.

Date Contract Initiated: Tracor Jitco subcontract initiated May 1, 1974; successor to NCI contract NO1-CP-23254 initiated June 12, 1970.

Current Annual Level: $210,083
227,590 (13 months)

2. Subcontract with Gulf South Research Institute

Title: Carcinogenesis Bioassay of Chlorinated Hydrocarbons, Organophosphates and Miscellaneous Pesticides

Contractor's Project Director: Dr. Harry Burchfield

Project Officers (NCI): Dr. Herman Kraybill
Dr. Thomas Cameron

Project Officers (Tracor Jitco): Dr. Carl J. Wessel
Dr. Donald Willigan

Objectives: To evaluate the carcinogenicity of 27 pesticides: Series I consists of chlordane, heptachlor, lindane, toxaphene, chlordecone, dieldrin, aldrin, endrin, photodieldrin, malathion, parathion, azinphosmethyl, dichlorvos, phosphamidon, dimethoate, Gardona^{R}, captan, daconil, picloram, and amiben. Series II consists of malathion, fenthion, coumaphos, aldicarb, dyrene, fluometuron, diazinon, and malaoxon. Three positive controls were assigned for Series I and consist of: 2-AAF, safrole and amitrole. No positive controls are assigned to the second series.

The protocol for Series I entailed the feeding of the pesticides for 80 weeks to both sexes of Osborne-Mendel rats and B1C3F1 mice at two dose levels, i.e.,

the estimated MTD and 1/2 MTD, using a group size of 50. Mice were sacrificed at 90 weeks and rats at 110 weeks. For Series II, a similar protocol is used except that F/344 rats and B6C3F1 mice are exposed for 24 months following which time they are sacrificed.

Major Findings: Series I compounds were put on test during the period of August, 1971 through September, 1972 and taken off test during the period of August, 1973 through October, 1974. The estimated maximum tolerated dose (MTD) was determined by six-week feeding studies. In no case was the dose level tolerated and all doses had to be reduced during the test because of toxic effects. In all, 175 dose changes were made. In some cases the final MTD was only 10% of the initial MTD. However, it is felt that initial use of 25% of the MTD as determined by six-week studies would have been satisfactory. Animals from the Series I chronic tests have now been sacrificed and their tissues are being studied by pathologists.

The Series II compounds (except malaoxon) have been procured and 90-day toxicity studies are underway to establish the MTD doses. Malaoxon, a metabolite of malathion, was not available off the shelf and required synthesis.

Significance to Biomedical Research and the Program of the Institute: Many of the pesticides under study have widespread usage resulting in extensive human exposure. The chemicals selected for testing have either been tested inadequately for carcinogenicity in the past or the results of such tests have been equivocal. These extensive experiments are designed to yield definitive data and help resolve these issues.

Proposed Course: Pathology will be completed and reports prepared on the Series I pesticides. The new compounds (Series II) are scheduled to be on chronic test by April 1975.

Date Contract Initiated: Tracor Jitco subcontract initiated November 1, 1974; successor to NCI contract NO1-CP-43267 initiated June 15, 1970.

Current Annual Level: NCI - $244,365 (5 months)
T.J.- $337,662 (7 months)

3. Subcontract with Hazleton Laboratories, Inc.

Title: Carcinogenesis Bioassay of Pesticides and Other Environmental Chemicals

Contractor's Project Directors: Dr. Marcelina B. Powers
Dr. Richard W. Voelker

Project Officers (NCI): Dr. Norbert P. Page
Dr. Elizabeth K. Weisburger

Project Officers (Tracor Jitco): Dr. Carl J. Wessel
Dr. Charlie N. Barron

Objectives: To determine the carcinogenicity of pesticides and other environmental chemicals in Osborne-Mendel and Fischer 344 rats, B6C3F1 and ICR Swiss mice, and Syrian golden hamsters by various routes of administration for a period of 18 to 24 months. There are 46 chemicals, grouped in 3 series, being investigated for carcinogenic potential.

Series A consists of 17 pesticides administered in the feed to B6C3F1 mice (90 weeks) and Osborne-Mendel rats (110 weeks): aminotriazole, chlorobenzilate, Bayluscide(R), DDE, p,p'-DDT, dioxathion (Delnav(R)), endosulfan (Thiodan(R)), kelthane (Dicofol), methoxychlor, nitrofen, pentachloronitrobenzene (PCNB), rothane (DDD), Zectran (R), safrole, trifluralin (Treflan (R)), vegadex (CDEC), and N-2-fluorenylacetamide (2-AAF).

Series B consists of 18 halogenated environmental chemicals administered by gavage to B6C3F1 mice (90 weeks) and Osborne-Mendel rats (110 weeks): 1,1,2,2-tetrachloroethane, chloroform, chloropicrin, ethylene dibromide, dibromochloropropane, ethylene dichloride, 1,1-dichloroethane, trichloroethylene, sulfolene, iodoform, 1,1,2-trichloroethane, tetrachloroethylene, hexachloroethane, carbon disulfide, carbon tetrachloride, methylchloroform, 3-chloropropene, and trichlorofluoromethane.

Series C consists of 11 chemicals recently added to the program: benzoin, titanium dioxide, dl-menthol, dimethyl terephthalate, sulfisoxazole, selenium disulfide, bis-(2-chloroisopropyl) ether, Selsun(R), ethylene dibromide, dibromochloropropane, and phenol. The duration of the chronic experiments on these chemicals will be 24 months in all species. The routes of dosing are in the feed (chemicals 1 through 4), by gavage (chemicals 5 through 7), in the drinking water (chemical 11), by cutaneous painting (chemicals 6 and 8), and by inhalation (chemicals 9 through 11). Both Fischer 344 rats and B6C3F1 mice are used for chemicals given orally and for the first 2 chemicals given by inhalation. ICR/Swiss mice only are used for cutaneous painting. Syrian golden hamsters only are used for the inhalational studies of phenol.

Major Findings: Of the 46 chemicals under study, dosing has been completed in rats and mice for 35 (Series A and B), and the animals have been killed and autopsied. Histopathologic evaluation of the tissues has been completed for the rats for 16 of these chemicals and for the mice for 18 of these chemicals and is in progress for the remainder.

Of the 11 chemicals in Series C, chronic oral studies have been started in rats and mice for 2 (titanium dioxide and dl-menthol) and in mice for another (benzoin). The 90-day subchronic studies have been completed in rats and mice for sulfisoxazole and selenium disulfide. Inhalational 90-day subchronic studies in rats and mice have been completed for ethylene dibromide and dibromochloropropane and levels of exposure established for the chronic studies. Preliminary irritational and toxicologic dermal studies are in progress. There are problems due to systemic toxicity and dermal irritancy.

Two industrial chemicals with agricultural use as fumigants, ethylene dibromide and 1,2-dibromo-3-chloropropane, were found to be highly active

carcinogens by gavage in Osborne-Mendel rats and B6C3F1 mice. Both chemicals were associated with a high incidence of squamous cell carcinoma of the stomach with extension to adjacent abdominal organs and metastases. The latter chemical was also associated with an increased incidence of mammary carcinoma in female rats.

Significance to Biomedical Research and the Program of the Institute: The chemicals under study have widespread usage and in many instances are components of products to which the human population is extensively exposed. If any of these chemicals are found to be carcinogenic, measures to control and to minimize human exposure and to assess possible significance of human exposure would be appropriate. Information gathered from these studies will also be valuable in establishing precautionary measures for handling and using these chemicals and for planning future investigations.

Proposed Course: Histopathology will be completed for the remaining pesticides and environmental chemicals. Evaluation of additional chemicals utilizing Fischer 344 rats and B6C3F1 mice for oral administration and ICR Swiss mice for multiple dermal exposures are in progress. The duration of exposure for these chemicals will be 24 months. Chronic (24-month) inhalational exposures of rats and mice to the two fumigants ethylene dibromide and 1,2-dibromo-3-chloropropane are scheduled. Preliminary inhalational studies on the toxicity of phenol in hamsters are also scheduled to establish levels of exposure for subsequent chronic studies.

Date Contract Initiated: Tracor Jitco subcontract initiated February 15, 1975; successor to NCI contract NO1-CP-43245 initiated December 1, 1970.

Current Annual Level: NCI - $526,255 (8 1/2 months)
T.J. $379,868 (3 1/2 months)

4. Subcontract with IIT Research Institute

Title: Carcinogenesis Bioassay of Chlorinated Dibenzodioxins and Chemicals

Contractor's Project Director: Dr. Maurice E. King

Project Officers (Tracor Jitco): Dr. Carl J. Wessel
Dr. Stephen S. Olin

Objectives: To determine the carcinogenicity and co-carcinogenicity of chlorinated dibenzodioxins and related compounds by application to the skin of mice and by oral administration to mice and rats. The toxic chemicals, Tetra-, Hexa-, and Trichlorodibenzodioxin (TCDD, HCDD, and TriCDD) are being administered by oral intubation (3 dose levels) in both Osborne-Mendel rats and B6C3F1 hybrid mice and by skin application (1 dose level, complete and initiated with DMBA) in Swiss-Webster mice. Other less toxic compounds tested in O-M rats and B6C3F1 mice at 2 dose levels are Dibenzodioxin, Dichlorodibenzodioxin, and Octachlorodibenzodioxin in the diet, and p-Dioxane in the drinking water.

Major Findings: TCDD, HCDD, and TriCDD have been synthesized in sufficient quantities and suitable purity for toxicity and carcinogenicity trials. Prechronic oral intubation studies of TCDD and HCDD clearly established the delayed toxic effects and cumulative toxicity of these compounds. Because of the markedly delayed manifestation of toxicosis, it was difficult to establish the LD50 after single doses, and indications of cumulative toxicity in the subchronic studies precluded the determination of the MTD in certain experiments. For both compounds, length of treatment is as important as the dosage regimen, because of the cumulative toxicity noted in 13-week subchronic studies. In general, female mice were more susceptible to hepatotoxic effects of the chlorinated dibenzodioxins than males. The differential susceptibility of sexes was not observed in rats. The chronic bioassays, including histopathological examination and evaluation, of the less toxic compounds are nearing completion.

Significance to Biomedical Research and the Program of the Institute: Some chlorinated dibenzodioxins are extraordinarily toxic contaminants in certain pesticides and other environmental chemicals. In addition to their extreme toxicity, they are highly stable under environmental conditions and may tend to accumulate in the food chain. They have been tested for acute toxicity and ratogenicity but have not been examined for carcinogenicity. This program is designed to accomplish the latter and is part of the NCI program of testing chemicals to which man is exposed for carcinogenicity.

Proposed Course: Chronic carcinogenesis bioassays of TCDD, HCDD and TriCDD will be initiated. These proposed long-term experiments should require three more years for completion.

Date Contract Initiated: Tracor Jitco subcontract initiated May 1, 1974; successor to NCI contract NO1-CP-12338 initiated June 25, 1971.

Current Annual Level: $316,975

5. Subcontract with Litton Bionetics, Inc.

Title: Carcinogenesis Bioassay of Environmental Chemicals

Contractor's Project Director: Dr. F. M. Garner

Project Officers (NCI): Dr. Norbert Page
Dr. Elizabeth K. Weisburger

Project Officers (Tracor Jitco): Dr. Carl J. Wessel
Dr. Stephen S. Olin

Objectives: To assess in rats and mice the carcinogenicity and chronic toxicity of selected chemicals present in man's environment. Secondary objectives may deal with the pathogenesis of cancer induction. In this program, the following chemicals have been administered to B6C3F1 hybrid mice and Fischer 344 rats, in most cases in the diet: 4,4'-Methylenebis-(N,N-dimethylaniline; 4,4'-Bis(N,N-dimethylamino)benzophenone; 1-Phenyl-2-thiourea;

Ethylenediamine tetraacetic acid; Dibutyltin diacetate; Styrene; β-Nitrostyrene; 3,3'-Dimethoxybenzidine diisocyanate; 2-Nitro-p-phenylenediamine; p-Chloroaniline; 3-Chloro-p-toluidine; 5-Chloro-o-toluidine; 2,4-Dichlorophenyl-4-nitrophenylether; p-Aminodiphenylamine; p-Nitrosodiphenylamine; 2,4-Dimethoxyaniline; Nitrilotriacetic Acid; Nitrilotriacetic Acid, trisodium salt; N-2-Fluorenylacetamide (Positive Control); Amitrole (Positive Control); 4-(Chloroacetyl)-acetanilide; Trimethylphosphate; Triphenyltin hydroxide; 1-Ethyl-3-(5-nitro-2-thiazolyl)urea; α-Bromo-α-ethybutyrylcarbamide; N,N-Diethylthiourea; Succinic acid mono-(2,2-dimethylhydrazide); 3-(Chloromethyl) pyridine HCl; Sodium iodomethane sulfonate; p-Quinone dioxime; 2-(Chloromethyl)pyridine HCl; p-Phenylenediamine dihydrochloride; 4-Nitro-o-phenylenediamine; 1-Phenyl-3-methyl-5-pyrazolone; 4-Amino-2-nitrophenol; Lithocholic Acid; Pivalolactone; 1,1,3-Trimethyl-2-thiourea.

Major Findings: Of the 42 chemicals under study, dosing has been completed in mice for 18 and in rats for 19. All of these animals have been killed and necropsied, and the histologic review of the tissues is in progress. A high incidence of thyroid adenomas and thyroid carcinomas was observed in Fischer rats given 1,1,3-Trimethyl-2-thiourea by gavage for 18 months. A spectrum of liver lesions, including hyperplasia, hepatic cell adenoma, and hepatocellular carcinoma, was induced in Fischer rats by administration of 4,4'-Bis(N,N-dimethylamino)benzophenone in the diet.

Significance to Biomedical Research and the Program of the Institute: By identifying potentially hazardous substances present in the environment, using the mouse and rat as a model, it may be possible to minimize man's exposure by prohibiting or restricting the use of these agents or modifying the method in which they are processed and handled.

Proposed Course: Eighteen chemicals will have the pathology of their animals completed and the results reported. The remaining 24 chemicals have animals still on test. The tests will be completed, with the kill, necropsy, pathology, and reporting to follow.

Date Contract Initiated: Tracor Jitco subcontract initiated August 1, 1974; successor to NCI contract NO1-CP-43349 initiated May 13, 1971.

Current Annual Level: NCI - $123,690 (2 months)
T.J. - $845,219 (10 months)

6. Subcontract with Mason Research Institute

Title: Carcinogenesis Bioassay of Environmental Chemicals

Contractor's Project Director: Dr. Emil Smith

Project Officers (NCI): Dr. Thomas Cameron
Dr. Elizabeth K. Weisburger

Project Officers (Tracor Jitco): Dr. Carl J. Wessel
Dr. Miles Davis

Objectives: To evaluate the carcinogenicity of 54 chemicals or combinations of chemicals, grouped into four series. Series I consists of twelve chemicals: Dulcin; Sodium nitrite; Adipamide; Acetamide; Urea; L-Arginine glutamate; n-Butyl urea; Hexanamide; p-Tolyurea; Dulcin + L-arginine glutamate; n-Butyl urea + Sodium nitrite; 2-AAF. Series II consists of twenty chemicals: N,N-Dimethyl-p-nitrosoaniline; 2,5-Toluene diamine sulfate; 5-Nitro-o-toluidine; Hydrazobenzene; 2,4-Dinitrotoluene; 2-Aminoanthraquinone; 3-Amino-4-ethoxyacetanilide; 3-Amino-9-ethylcarbazole; 1-Amino-2-methylanthraquinone; 6-Nitrobenzimidazole; 2-Methyl-1-nitroanthraquinone; 5-Nitro-o-anisidine; 4-Nitroanthranilic acid; 1-Nitronaphthalene; 5-Nitroacenaphthene; 3-Nitro-p-acetophenetide; 2,4-Diaminoanisole sulfate; 3-Amino-1,2,4-triazole; Aspirin-phenacetin-caffeine (APC); 2-AAF. Series III has eighteen chemicals: p-Cresidine; m-Cresidine; 2,5-Dithiobiurea; p-(Dimethylamino)benzene diazo sodium sulfonate; 1,5-Naphthalene diamine; 4-Nitro-2,3,5,6-tetrachloranisole; Cupferron; Diaryl anilide yellow; tris(2,3-Dibromopropyl) phosphate; N-(1-Naphtyl)ethylene diamine dihydrochloride; 4-Chloro-o-phenylene diamine; 4-Chloro-m-phenylene diamine; 2-Chloro-o-phenylene diamine sulfate; 1,2,3-Benzotriazole; Aniline hydrochloride; o-Anisidine hydrochloride; p-Anisidine hydrochloride; 2-AAF. Series IV consists of four chemicals: Cinnamyl anthranilate; 2-Amino biphenyl; 4-4'-Oxydianiline; 2,6-Toluenediamine. For series I, II, and III, 2-AAF is a positive control, and 3-Amino-1,2,4-triazole is a positive control for series II. Negative and vehicle (acetone and corn oil) controls are also run.

The chemicals are being mixed in the diet at two levels, the estimated maximum concentration consistent with prolonged survival of the animals and one-half of this concentration. Food containing these levels of each chemical are being fed for 18 or 24 months in groups of Fischer 344 rats and C57BL/6Cr or B6C3F1 mice. Groups consist of 50 males and 50 females. When treatment is terminated at 18 months, five males and five females are sacrificed at that time. The surviving mice are maintained for an additional three months on a chemical-free diet before sacrifice and the surviving rats are sacrificed after an additional six months on a chemical-free diet. In experiments involving treatment for 24 months, all animals are sacrificed at the end of treatment.

All animals are being observed daily for signs of chemical toxicity and tumor development. In addition, body weight and food consumption is being measured at regular intervals. Animals which are moribund or which die and all animals which are sacrificed receive extensive gross and microscopic examination, including a detailed examination of gross lesions and tissue masses and all major organs.

Major Findings: All animals treated with chemicals of series I have been necropsied and histopathologic evaluation of the tissues of these animals is underway. Studies of some of the chemicals in series II have progressed to the point where histopathologic evaluation is being done, while for other compounds the animals are still being treated or observed following the course of treatment. Studies on all series III compounds have started and animals are being treated with these substances or are being observed after treatment. Series IV chemicals are in pre-chronic testing.

Significance to Biomedical Research and the Program of the Institute: One of the functions of the Carcinogenesis Program is to evaluate the potential carcinogenic risk that is represented by chronic exposure to environmental chemicals. This contract will be significant in identifying and minimizing potentially hazardous substances to which man is exposed.

Proposed Course: Bioassay experiments will be continued through the chronic tests, sacrifice, necropsy, pathology, data reporting, and preparation of Technical Reports.

Date Contract Initiated: Tracor Jitco subcontract initiated December 29, 1974; successor to NCI contract NO1-CP-43297 initiated May 1, 1971.

Current Annual Level: NCI - $386,717 (7 months)
T.J. -$324,171 (5 months)

7. Subcontract with Midwest Research Institute

Title: Analytical Chemistry Resource

Contractor's Project Director: Dr. Evelyn Murrill

Project Officers (Tracor Jitco): Dr. Carl J. Wessel
Dr. Stephen S. Olin

Objectives: MRI serves as an analytical resource for the bioassay Prime Contract. Specific tasks include analysis of chemicals for identity, assay, and stability; formulation of protocols for chemical mixes; analysis of feed samples for toxic components; and analysis of dosed-feed samples. Special tasks include isolation and identification of impurities and other analytical problems.

Major Findings: Since June, 1972, studies have been completed and reported on 189 batches of chemicals (100 different chemical substances). Twenty have been analyzed in detail to identify and quantitate minor impurities, and 62 chemical mixes have been analyzed for stability or homogeneity. Routine characterization studies include chromatography (TLC, GC, HPLC, etc.), elemental analysis, water analysis, and other assay methods, dependent on the chemical (titration, voltammetry, mass spectrometry). Physical data include transition data (melting point, boiling point), refractive index, optical rotation, and spectral data (infrared, ultraviolet, visible, and NMR). Chemical/feed mixtures have been analyzed at zero time and after storage intervals by high-pressure liquid chromatography, voltammetry, or reduction combined with ultraviolet analysis. Preparative high-pressure liquid chromatography and GC/MS have been used with some of the techniques listed under routine analysis for solving impurity and purification problems.

Significance to Biomedical Research and the Program of the Institute: The literature of carcinogenesis bioassay studies contains many examples of experiments which cannot be meaningfully interpreted because the test

substances had not been adequately characterized chemically. When such an experiment results in low tumor incidence, for example, the question will always remain as to whether the tumors observed might not have arisen from small concentrations of highly potent impurities, rather than from the allegedly weakly potent nominal ingredient. Other questions of a chemical nature which are central to the interpretation of bioassay data include such problems as decomposition of the chemical in feed or intubation mixtures and levels of mycotoxins or pesticides in food and bedding. For these reasons it is essential that the Carcinogenesis Program have available to it a facility capable of characterizing those materials entered into the testing program as well as determining the effectiveness of administration of these substances to the test animals.

Proposed Course: To continue to serve as an analytical resource for the bioassay program by homogenization and analysis of bulk chemicals, analysis of vehicles, stability analysis of the bulk chemical and of chemical/vehicle mixtures, and by the preparation of protocols for obtaining homogeneous chemical/vehicle mixtures.

Date Contract Initiated: Tracor Jitco subcontract initiated July 1, 1974; successor to NCI contract NO1-CP-23270 initiated June 28, 1972.

Current Annual Level: $209,238

8. Subcontract with Papanicolaou Cancer Research Institute

Title: Bioassay of Potential Carcinogenic Compounds in Six Inbred Strains of Rats

Contractor's Project Director: Dr. Wilhelmina F. Dunning

Project Officers (NCI): Dr. James M. Sontag
Dr. Thomas P. Cameron

Project Officers (Tracor Jitco): Dr. Carl J. Wessel

Objectives: To determine the suitability of several inbred strains of rats for possible use in bioassay of chemicals for carcinogenicity. A comparison is being made of the response of each strain to the force-feeding of a chemical carcinogen.

Major Findings: Comparative chronic tests are almost completed with the chemical N-hydroxy-N-2-fluorenylacetamide in six inbred strains of rats. These include the Fischer line 344, A x C line 9935 Irish, August line 990, Marshall line 520, S x F line 40814 and Zimmerman line 61.

A total of 667 rats have been fed N-hydroxy-N-2-fluorenylacetamide on a chronic schedule. The females received 10.0 and 5.0 mg/kg by intubation on a 5-day-a-week schedule for six months. The males of each strain were given 5.0 and 2.5 mg/kg for a similar time. A total of 130 are still alive and under observation.

Most of the necropsy records are complete for 545 rats that survived at least six months of chronic feeding. A total of 275 induced neoplasms have been described, of which 269 have been confirmed and classified by microscopic examination. The most frequent site of neoplastic changes was the liver with 117 tumors of which 113 have been classified as malignant hepatomas or adenocarcinomas with a few cholangiomas. Multiple liver tumors were frequent. The liver tumors occurred in rats of each strain, more frequently in males than females at the higher dose levels. The squamous division of the stomach was the next most frequent site of neoplastic change. In this organ 95 neoplasms have been described and all have been confirmed to be squamous cell carcinomas. About 35 per cent of these tumors were observed in rats of the August line 990. In addition, 22 A x C line 9935 and S x F line 40814 rats were observed with squamous cell carcinomas of the face, orbit, or ear canal, and 12 with carcinomas of the urinary bladder. Tumors of the mammary gland, uterus, ovary, spleen, lung, and mesentric lymph nodes were less frequent.

Approximately 100 rats (16 of each strain) are serving as vehicle controls and another group of 100 as untreated controls. Nearly half of these rats are alive. Only six neoplasms have been observed to date.

Significance to Biomedical Research and the Program of the Institute: As part of the Bioassay Operations Segment's program, compounds are tested in only one or two different rat strains. As an outcome of this study, more suitable strains of rats than those presently in use may be found. This would facilitate the bioassay testing program.

Proposed Course: Follow those animals that are still alive and complete the necropsy and pathology reports for those that have died or have been sacrificed.

Date Contract Initiated: Tracor Jitco subcontract initiated February 10, 1975; successor to NCI contract NO1-CP-23253 initiated June 26, 1972.

Current Annual Level: NCI - $49,000 (8 1/3 months)
T.J. $21,915 (3 2/3 months)

9. Subcontract with Southern Research Institute

Title: Carcinogenicity Studies of Chemotherapeutic Agents and Related Chemicals

Contractor's Project Directors: Dr. D. P. Griswold
Dr. John Peckham
Dr. David Prejean

Project Officers (NCI): Dr. Norbert P. Page
Dr. Elizabeth K. Weisburger

Project Officers (Tracor Jitco): Dr. Carl J. Wessel
Dr. Garth Fitzhugh

Objectives: To evaluate the potential carcinogenicity of chemotherapeutic agents and related chemicals in rats and mice following long-term exposure.

Three series of studies have been in progress. In the first series, carcinogenic trials consisted of a 12-month injection or gastric intubation period and a subsequent 6-month observation period. These compounds were administered on a 3x/week schedule. Compounds included in this first series were myleran, thio-TEPA, emetine, dibenzyline, B-deoxythioguanosine, 1-propanol, 3,3'-iminodidimenthane sulfonate(ester) HCl, 5-azacytidine, isophosphamide, ICRF-159, methanesulfon-m-anisidide, 4'-(9-acridinylamino)-, monohydrochloride (MAAM), and acronycine by injection, and phenesterin and estradiol mustard by intubation. Procarbazine was given by injection as a positive control.

A second series of compounds was started on 5-day per week feeding schedule for 18 months, after which a 6-month observation period followed before final sacrifice. As with the first series, two dose levels, an MTD and a 1/2 MTD, were employed for each compound with 35 male and 35 female animals at each dosage level. The compounds in this series were o-anthranilic acid, phenazopyridine, pyrimethamide, 4,4'sulfonyldianiline, L-tryptophane, phenformin, tolbutamide, chlorpropamide, 4,4'-thiodianiline, ethionamide, pyrazinamide, tolazamide and acetohexamide. N-2-fluorenylacetamide was used as a positive control. On November 20, 1974, a subcontract modification extended all evaluations still in the treatment stage to about 24 months; or specifically 110 weeks for rats and 104 weeks for mice.

Those compounds administered by intubation or injection are being evaluated in Sprague-Dawley rats and B6C3F1 mice. Fischer rats and B6C3F1 mice are being used for those administered in the diet.

A third series of compounds will follow the protocol outlined in the "Guidelines for Carcinogenic Bioassay in Small Rodents" except that treatment will be 3x/week rather than daily in those agents administered by injection. Because some chemotherapeutic agents suppress the bone marrow and cause early death, additional animals have been added in the subchronic study to determine the degree of myelo-suppression caused by each compound. In this series adriamycin and hexamethylmelamine have been placed on the chronic bioassay and reserpine is in the subchronic phase.

Major Findings: Histopathological evaluation of the Sprague-Dawley rats and B6C3F1 mice exposed to procarbazine, myleran, isophosphamide, acronycine, 5-azacytadine, thio-TEPA and 1-propanol, 3,3'-iminodidimethane sulfonate (ester)HCl(dimethane sulfonate) are complete. Also complete are the evaluations on 4,4'-Sulfonyldianiline (dapsone) in Fischer-344 rats and B6C3F1 mice.

Nervous system neoplasms, primarily olfactory neuro-epithelial tumors, were seen in 30 percent of the rats, 28 percent of the male mice (high dose), and in 34 percent of the female mice (high dose) treated with procarbazine.

Lymphoblastic leukemia occurred in 71 percent of the high dose male mice and in 91 percent of the high dose female mice treated with thio-TEPA. The low

dose male mice also had a 44 percent incidence of squamous cell carcinoma. Dapsone-treated male mice (high dose) had a 37 percent incidence of peritoneal sarcomas.

Significance to Biomedical Research and the Program of the Institute: Results will provide clinicians with a basis for the selection of agents to be used in long-term chemotherapy in man.

Proposed Course: To complete bioassay procedures on the remaining 22 agents under evaluation in this contract.

Date Contract Initiated: Tracor Jitco subcontract initiated October 1, 1974; successor to NCI contract NO1-CP-33214 initiated June 24, 1968.

Current Annual Level: NCI - $103,664 (4 months)
T.J. - $376,182 (8 months)

10. Subcontract with Stanford Research Institute

Title: Combined Effects of Chemical Carcinogens and Other Chemicals

Contractor's Project Director: Dr. David C. L. Jones

Project Officers (Tracor Jitco): Dr. Carl J. Wessel
Dr. Miles Davis

Objectives: To investigate (1) the effect of mixtures of carcinogenic and noncarcinogenic chemicals on the induction of tumors when introduced into rodents by oral administration, (2) whether the interactions among groups of chemicals in acute and subacute toxicity experiments predict the effects of the same combinations in carcinogenicity experiments, and (3) the feasibility of conducting initial screening bioassays of chemicals in groups without thereby obscuring the carcinogenicity of any individual member of the groups.

Major Findings: Two year survivors of groups of Fischer 344 rats fed compounds in Group 1 (N-methyl-N'-nitro-N-nitrosoguanidine, N-butanol-N-butylnitrosamine, nitrilotriacetic acid, dipentylnitrosamine), Group 2 (aflatoxin B_1, cycad flour, lasiocarpine, dipentylnitrosamine), and most of Group 3 (aflatoxin B_1, N-butanol-N-butylnitrosamine, lead acetate, thiouracil) have been terminated and histologic evaluation of selected tissues is now in progress. Group 3 animals receiving thiouracil at the low level and Group 4 compounds (aflatoxin B_1, dieldrin, hexachlorophene, Aroclor 1254) are approaching two years on chronic test. Comparison among groups for evidence of interactions among compounds awaits completion of histologic evaluation.

Significance to Biomedical Research and the Program of the Institute: A major portion of the resources of the National Cancer Institute's Carcinogenesis Program is devoted to testing environmental chemicals for carcinogenicity. In these tests, the experimental protocols generally attempt to expose animals to one chemical while minimizing exposure to other chemicals.

There are two problems involved in this approach. First, in the human environment, men are exposed to multiple chemicals simultaneously and sequentially rather than to a single chemical. Second, resources are available to test only a small portion of the chemicals in our environment if they are tested singly. Newer chemicals are generated faster than the available resources are capable of testing each one by a protocol considered acceptable. This project is the beginning of a program that will investigate how mixtures of carcinogenic and noncarcinogenic chemicals interact to affect the induction of tumors and also to investigate the feasibility of conducting initial screening bioassays of chemicals in groups without thereby obscuring the carcinogenicity of any individual member of the group. The biological studies from this contract will be analyzed by mathematical statisticians working on a separate contract with the NCI to determine whether consistent mathematical models of interactions can be developed.

Proposed Course: Termination of the remaining chronic feeding groups will occur within the next six months. After completion of the histologic evaluation the results will be subjected to statistical evaluation and a final report prepared.

Date Contract Initiated: Tracor Jitco subcontract initiated June 1, 1974; successor to NCI contract NO1-CP-43272 initiated June 24, 1971.

Current Annual Level: $697,691

SUMMARY REPORT

Biological Models Segment

July 1, 1974 through June 30, 1975

The Biological Models Segment is responsible for developing experimental models for studying the etiology and pathogenesis of the major forms of human cancer. A further long-range objective is the subsequent use of these models for the development of methods to prevent or modify the induction and progression of these tumors. Separate segments exist for lung cancer and cancer of the colon and rectum, but the remaining types of human malignancy fall within the area of responsibility of the Biological Models Segment.

The forms of cancer chosen to be investigated are selected by the Segment with extensive scientific advice from outside expert consultants and from NCI Staff as well as from societal needs as voiced by legitimate spokesmen through established and other respected communication media. The development of the Segment program is synthesized by an evaluation of current activities and their impact on program goals, and on new or specific ideas offered. The feasibility of initiating new areas of investigation has resulted from advances made in new research concepts and methods. After priorities are established, the degree of achievement of the Segment program is determined by available resources and, ultimately by the acumen provided by investigators participating in this endeavor, both within the collaborative projects and the NCI staff.

The Segment program presently includes a total of 29 active contracts covering areas of carcinogenesis studies with the pancreas, prostate, mammary gland, liver, kidney, skin, as well as studies on perinatal cancer in vitro epitheleal carcinogenesis, and some other areas. Investigations are not limited to the use of whole animals, though this is perhaps the greatest emphasis of the program. Cell and organ culture studies are specifically designed to develop the appropriate methodology for long-term maintenance of tissue in as near a normal state as possible for use as a tool for defining early stages of the carcinogenic response in target cells.

The responsibilities of Director of this Segment have continued to be held by Dr. Richard Pledger who also maintained his roll as Segment Manager. Staff from the Carcinogenesis laboratories have served as project officers and reviewers. The potential of the Biological Models Segment to administer a viable and active program was enhanced this past year by the addition of Dr. Thomas P. Cameron as a working member of the Segment. The major emphasis of the Segment on animal carcinogenesis studies requires a constant evaluation and improvement of veterinary procedures and animal housing techniques in order to maximize the health of the laboratory species being used and to assist in the interpretation and impact of new developments on the overall program.

Pancreas Carcinogenesis

With organization of the Segment in 1971, the initial and greatest effort was expended in developing a rational approach to carcinogenesis of the pancreas. Work has been highly productive since that time. This fiscal year has seen the fruition of the original research plans, namely the generation of animal models of pancreatic cancer. A number of approaches are now supported, each at different stages of development, and each with their own individual merits.

As was reported last year, from the University of Kansas Medical Center, (NO1-CP-23271) a 36% incidence of pancreatic adenocarcinoma was induced in the inbred strain 13 guinea pigs after weekly gavage administration of methylnitrosourea. In random-bred guinea pigs, after 15 months of a similar dosage regimen, no tumors have been observed. This result has been independently confirmed by similar investigations at Microbiological Associates, Inc. (NO1-CP-02199). The only alternations observed in the random-bred colony were variable degrees of atrophy of the pancreas and an occasional mitotic figure in the ductal epithelial lining.

Studies at Dartmouth College (NO1-CP-33378) have demonstrated carcinogenicity of azaserine for the pancreas of rats receiving one or two weekly injections intraperitoneally for six months. During the first year nodules of atypical exocrine cells representing hyperplastic foci and encapsulated adenomas were observed. After one year, carcinomas were seen in the pancreas of more than 25% of the treated animals. Such glands can weigh up to 4 grams. Metastases were observed in lymph nodes, mesenteric fat, liver and lung. This tumor appears at this stage to originate from acinar cells in contrast to the hamster model discussed below. In addition to azaserine, a nitroso derivative of serine, an amino acid known to concentrate in the rat pancreas, was chemically synthesized. O-(N-methyl-N-nitroso-beta-alanyl)-L-serine has a similar tissue distribution as azaserine and similarly induces nodules of atypical acinar cells during the first year of treatment in greater frequency than in controls. Further time is required to determine if carcinomas will develop as was observed with azaserine.

In a related project, supported through the Office of the Associate Director rather than through this Segment, and primarily devoted to the study of organotropic effects of N-nitroso compounds, the Eppley Institute for Cancer Research, University of Nebraska, (NO1-CP-33278) has recently reported a high incidence of pancreatic neoplasms in Syrian golden hamsters following subcutaneous administrations of diisopropanol-nitrosamine once weekly for life. Tumor latency was as short as 15 weeks, and the incidence approached 100% particularly in the males. Histologically, these tumors were of ductal origin resembling those neoplasms of humans. This most interesting model offers a unique opportunity for study of pancreas carcinogenesis by an indirect route of carcinogen administration. Other possible beta-oxidation metabolites of di-n-propylnitrosamine failed to yield similar results. These observations obtained during the past year, have a significant impact on the development of the pancreas carcinogenesis program.

Other approaches have been attempted by investigators with promising results. Although it is premature to describe these observations in depth, suffice it to say that apparently tumors can be induced with methylnitrosourethan upon constant application directly and solely to the ductal cells by intraductal catherization in rabbits (Boston University, NO1-CP-23297). This latter observation is undergoing confirmation at the present time. Other projects relating to the pancreas continue to be supported on organ (University of Tennessee, NO1-CP-33369) and exocrine cell culture (Wright State University, NO1-CP-43231). These difficult studies attempt to utilize the latest in vitro techniques to provide a research tool to the Program. Case Western Reserve University (NO1-CP-23284) has shown alkylation of pancreatic RNA and DNA by methylnitrosourea and methylnitrosourethan. In vivo studies indicate enhanced thymidine uptake in the duodenal pancreas and opposed to the mid or splenic segments, somewhat consistent with the exocrine tumors induced by carcinogens. These and other studies on the pancreas have reached a point that for the first time the Carcinogenesis Program was able to sponsor a half-day conference on Pancreatic Carcinogenesis, held on February 4, 1975, during the Third Annual Carcinogenesis Collaborative Conference. Coordinated publication of these advances in a scientific journal has been arranged, and it is hoped that it will stimulate other investigators in various fields of research to participate in this significantly advancing field of carcinogenesis research.

New projects in pancreatic carcinogenesis are scheduled for initiation this fiscal year, dealing principally with chemical structural moieties which tend to concentrate in this organ, and with evaluation of mechanisms by which carcinogens can preferentially induce pancreatic cell alterations in man and animals. The two likely routes con idered feasible are reflux of bile or duodenal constituents containing carcinogenic substances into the pancreatic duct, or general distribution via the blood stream of cancer-inducing substances which have a tissue specificity for pancreatic cells.

Prostate Carcinogenesis

Attempts to induce consistently cancer of the prostate in various laboratory animals has been pursued for almost 40 years with minimal success. This exceedingly difficult problem is a constant challenge to the Segment, and a number of activities have been initiated to explore new opportunities developed by the research community.

It was reported last year that work at the Southwest Foundation for Research and Education (NO1-CP-33379) demonstrated a significant change in both the pattern of metabolic activity of testosterone and the number of androgen cytoplasmic and nuclear binding sites in the prostate of different aged male rats. This work has been extended during the past year with a number of new unique observations: 1) Histologically confirmed spontaneous prostatic adenocarcinoma was observed in 6 out of 41 AxC conventionally housed rats surviving 34 to 37 months. Grossly, the lesions appeared as small hemorrhagic foci, confined solely to the ventral prostatic lobe. 2) The ratio of reduced (17-hydroxy) endproducts of testosterone metabolism by rat ventral prostatic tissue to oxidized substances (17-keto) decreases from a maximum of 5.1:1 to 1.3:1 as the donor rat age is advanced from 90 days to 36 months.

3) There exists an aging -associated reduction in the number of cytoplasmic and nuclear androgen receptor content of the ventral prostate. Such reduction is consistently detectable in three strains of rats, Sprague-Dawley, Royalhart and AxC. 4) In male dogs, in complete contrast to the rat, testosterone metabolism is observed in beagles to be predominantly and strikingly oxidative. The extent of this oxidative capacity is shifted to even further oxidative metabolism using hyperphysiological levels of androgen. Similarly, in complete contrast to the rat, aging in the dog results in a very pronounced shift to reductive metabolism. Measurements of canine receptor sites have not been performed, and thus no comparison with the rat can be made. Similar but less significant differences are observed with the dorsolateral lobe of the rat prostate. There is as yet no significance to results using empirically selected dorsal, ventral or lateral segments of the canine prostate.

These results suggest that the changing endocrinology of the aging rat prostate is intimately associated, with, and perhaps predisposing to, the development of adenocarcinoma. The exclusivity in aged rats of adenocarcinoma to the ventral prostate only; the shift in metabolism of testosterone, most pronounced in the ventral lobe; and the reduction of androgen receptors in the ventral lobe, somewhat greater than in other lobes and associated with a significant change in receptor distribution between the nuclear and cytoplasmic compartment, tend to foster a greater evaluation of the changing endocrinology of the aging animal, particularly with respect to the contrasting canine metabolic processes and the very high occurrence of benign hyperplasia in this latter species.

A workshop on prostate carcinogenesis was held during this year in which it was clearly indicated that insufficient information is available on normal human prostate and its aging. It is clear that this must be developed if emerging models are to be used with proper validity to human prostatic cancer. The resource base for this program is being expanded by the planned award of a contract by which two strains of rats will be aged for use in studies on prostate carcinogenesis.

Mammary Gland Carcinogenesis

It was previously reported that studies at the University of Nebraska at Lincoln (NO1-CP-33289) had succeeded in achieving nodular "transformation" of mammary epithelium in organ culture by a chemical carcinogen within 2-3 weeks. Mammary glands of young female mice in culture treated with DMBA or MCA during hormonal induction of alveolar growth produced nodules morphologically similar to hyperplastic alveolar nodules produced in vivo. Frequently, the in vitro nodules exhibit sustained milk-like secretory material. The transplantability of these lesions back to normal and hormonally stimulated, isogeneic hosts is being performed. Numerous solid lesions appearing hyperplastic or metaplastic in outgrowth of ductal epithelium, some with disorganized architecture, was observed. Additional work is in progress to determine conditions for appropriate outgrowth and assessment of the significance of such structures.

Research at the Energy Research and Development Administration's Brookhaven National Laboratory (Y01-CP-30213) has confirmed, using neutrons, the previously reported synergistic interaction of irradiation and estrogen in the induction of mammary tumors, first shown to occur with X-rays and estrogen by Dr. A. Segaloff of Alton Ochsner Medical Foundation (N01-CP-43229). This synergism occurs only with inbred AxC female rats and not the random-bred Sprague-Dawley females. This strain difference to the sensitivity of estrogen is significant, as is the genetic influence of radiation or DMBA on the increased induction of mammary tumors in the latter strain.

Other Activities

Many observations have been made which should eventually provide a meaningful base upon which additional studies will be developed. Completion of studies with N-4-4'fluorobiphenyl-acetamide induced kidney tumors in rats is expected to offer a morphologic model of human renal carcinoma. Perinatal and in vitro carcinogenesis projects have been initiated, and it is anticipated that a study to assess the rhythmic alterations of levels of certain hormones in women from the United States and Japan will be initiated. This latter program has principal relevance in the Segment's long-range program plan in mammary gland carcinogenesis research.

Small workshops continue to be a significant and productive mechanism to assess the status of program development and to define appropriate research opportunities for investigation. These have occurred within the larger pancreas and prostate programs, but it has become increasingly evident that other areas of Segment involvement are developing to a stage in which such in-depth assessment would be a productive adjunct to the normal planning sequence.

Interaction continues with similar program groups such as the National Organ Site Programs Branch of the Institute. In particular, the two major Segment efforts in carcinogenesis of pancreas and prostate are developed with direct interaction with the new National Pancreatic Cancer Project and the existing National Prostatic Cancer Project. Through such contact each program area augments relevant studies based upon immediate assessment of the implication of results irrespective of the source of project support.

CONTRACT NARRATIVES

BIOLOGICAL MODELS SEGMENT

July 1, 1974 through June 30, 1975

ALTON OCHSNER MEDICAL FOUNDATION (NO1-CP-43229)

Title: Carcinogenesis by Radiation plus Estrogen

Contractor's Project Director: Dr. Albert Segaloff

Project Officers (NCI): Dr. Norbert Page
Dr. D. Jane Taylor

Objectives: To investigate interaction or synergism of radiation and hormones in the induction of mammary tumors.

Major Findings: The contractor has been able to show that a single dose of X-ray delivered to one mammary chain of A x C inbred rats which had also received a continuous exogenous source of diethylstilbestrol (DES) resulted in a significant increase in mammary tumors over that resulting from the estrogen alone. None of the non-estrogen treated control animals developed cancers. Those which were radiated alone developed cancers at a much later time. There is a dosage response between radiation in the range used (50, 150 or 450 r) and tumor induction in the DES-treated rats. The greatest synergism was attained with 150 r. Although in intact female A x C rats continuous administration of progesterone will inhibit both estrogen-induced mammary cancer and radiation synergism (800 r), it does not inhibit estrogen-induced mammary cancers in the absence of the ovaries. In intact female A x C rats mammary cancers induced by DES generally show regression after oophorectomy, even though the DES pellets are still in place. Studies are now under way to test the effect of pregnancy, its timing, and its hormonal changes upon the synergism.

Significance to Biomedical Research and the Program of the Institute: Both radiation and ovarian hormones, given individually in large doses, have been implicated in causing an increase in breast cancer of both laboratory animals and women. It has been speculated that X-rays, routinely used for diagnosis and radiotherapy, plus estrogens and progesterones, now being widely used in birth control pills or for postmenopausal therapy, may interact synergistically and be contributing to the alarming rise in the incidence of breast cancer. In recent years, the Carcinogenesis Program of the NCI has devoted some efforts and funds to studies of the hormones, progestogens, and estrogens which are often combined in contraceptive pills. This study is an extension of efforts in this field.

Proposed Course: The radiation/estrogen studies are completed and being analyzed. Additional studies are under way to test the effect of early pregnancy which was observed as protection in human epidemiologic studies.

Estriol is being tested for its possible carcinogenic effect when given in the same manner as optimal DES.

Date Contract Initiated: May 25, 1971

Current Annual Level: $94,000

BIOTECH RESEARCH LABORATORIES, INC. (NO1-CP-55640)

Title: Endpoints for In Vitro Carcinogenesis Without Transplantation to Syngeneic Hosts

Contractor's Project Director: Dr. Robert C. Y. Ting

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: To define the parameters of assay (growth in soft agar, growth in factor-free medium, and cytotoxicity by activated macrophage) and to generate a usable protocol for detecting in vitro carcinogenesis (transformation) without the necessity for in vivo transplantation to syngeneic hosts.

Major Findings: (1) No significant differences have been found in the growth of normal and murine sarcoma virus (Kirsten) transformed rat embryo cells in factor-free medium. (2) Activated macrophages have been found to be cytotoxic to both normal hamster embryonic cells (early passage) and benzo[a]pyrene transformed hamster embryonic cells (established cell line). (3) A positive correlation between growth in soft agar and tumorigenicity has been established for early passage (4th and 8th) rat embryo cells transformed with Kirsten MSV. (4) Recent observations on cell aggregation differences in growth medium above an agar layer indicate the possibility of a new parameter for measurement of transformation. Rat embryo cells six days after MSV infection exhibit larger cell aggregates than cells from a normal counterpart population. An established line of benzo[a]pyrene transformed hamster cells has also been found to undergo cell aggregation. Studies will be conducted to ascertain if after BP treatment early passage normal rat and hamster embryonic cells exhibit larger aggregates, and to determine if such differences can be correlated with tumorigenicity.

Significance to Biomedical Research and the Program of the Institute: Any in vitro assay for transformation that can successfully predict tumorigenicity will be of great value in screening potential carcinogens. Such assays would also provide a means of monitoring in vitro transformation of human cells.

Proposed Course: Studies will be continued to evaluate and standardize the parameters of assay using early passage normal and transformed (viral and chemical) rat and hamster embryonic cells, and correlations with tumorigenicity in immunosuppressed animals will be established. Attempts will be made to clone and transform epithelial (liver) cells with subsequent transplantation experiments. The criteria derived from these rat and hamster cell systems will be used to test human fibroblastic and epithelial cells.

Date Contract Initiated: October 15, 1974

Current Annual Level: $121,143

BOSTON UNIVERSITY (NO1-CP-23297)

Title: Study of Chemically Induced Carcinogenesis of the Pancreas

Contractor's Project Director: Dr. Richard Elkort

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: To examine the techniques and effects of surgically implanting a carcinogen-containing permeation catheter into the mammalian pancreatic duct as a means of inducing adenocarcinoma.

Major Findings: A catheter delivery system has been developed which can be implanted directly into the main pancreatic duct of the New Zealand white rabbit without ligating the duct. Initial experiments were carried out with silicone polycarbonate catheters, but more recent studies have utilized nylon catheters, since this material markedly prolongs the duration of carcinogen exposure. Carcinogens under current study are 40% dimethylhydrazine (DMH), 0.05% benzo[a]pyrene (BP), and methylnitrosourethane (MNUT). Utilizing nylon catheters containing 10 microliters of carcinogen with release occurring over 20-40 days, successive histologic changes have been observed in the ductal cells of the DMH and BP series. Hyperplasia, dysplasia and metaplasia appeared at 14-18 weeks progressing to peri-ductal adenosis and adenoma formation at 34-48 weeks. The MNUT series is still under study but recent observations suggest that this agent produces more toxicity and atrophy than either the DMH or BP. A single observation has been made in the "re-implantable" catheter series in which a second carcinogen containing catheter replaces the initial catheter 30 days post-implantation. The first animal autopsied in this series 90 days post-implantation showed changes consistent with early, very well-differentiated carcinoma of the pancreas, probably of ductal cell origin. Currently, major efforts are being directed towards confirming this initial observation.

The specificity of the proposed model continues to be excellent, since all observed histologic changes are confined to the pancreatic ductal cells and surrounding pancreatic tissues. In addition, the slow, stepwise progression towards neoplasia and the small doses of carcinogen employed suggests that this model might closely approximate human pancreatic carcinogenesis.

Significance to Biomedical Research and the Program of the Institute: The establishment of an animal model for pancreatic adenocarcinoma is the prime goal of this contract, which represents one approach to reach this goal. If induction by this method can be confirmed, a detailed study of the neoplastic process and the mechanism involved in neoplastic transformation may then subsequently occur.

Proposed Course: Contractor will continue to determine the optimal concentrations of various carcinogens with which the ductal cells are to be challenged. An implanting device with properties which permit controlled diffusion of the encapsulated carcinogens so that the target cells are continually bathed with the inducing agent has been designed. Surgical implantation will be performed in rabbits for long-term studies. In addition, intensive efforts will be made to evaluate the effects of carcinogen augmentation via catheter re-implantation or intermittent refilling. At present, these approaches seem to represent the best method of achieving the primary goal of this program.

Date Contract Initiated: June 28, 1972

Current Annual Level: $42,000

CASE WESTERN RESERVE UNIVERSITY (NO1-CP-23284)

Title: Enhanced Induction of Guinea Pig Pancreatic Adenocarcinoma

Contractor's Project Director: Dr. Samuel S. Epstein

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: This project is designed primarily to investigate the carcinogenic potential on F_1 progeny of Hartley guinea pigs, of maternal transplacental exposure to NMU and NMUT and to investigate the carcinogenic effects of NMU and NMUT in male Hartley guinea pigs following partial pancreatectomy. Additional studies are performed to investigate the possible in vivo biosynthesis of NMU, and its carcinogenic effects, following methyl guanidine-sodium nitrite administration to guinea pigs and to examine the biochemical effects of NMU and NMUT-nucleic acid interactions.

Major Findings: A high incidence of still births and progressive mortality, associated with extensive fatty degeneration of liver, was observed in the F_1 progeny which received NMU in utero. There was no significant pre- or post-natal toxicity in the F_1 progeny of NMUT-treated or control maternal guinea pigs. Tumors have been induced in 4 male Hartley guinea pigs treated with NMU, of which one was an adenocarcinoma of pancreas.

Following in vivo administration of ^{14}C-labeled NMU and NMUT, the uptake of the label was higher in the duodenal than in the mid and splenic segments of pancreas. NMU and NMUT both alkylated pancreatic DNA and RNA, yielding 7-methylguanine. NMUT also produced 6-O-methylguanine in guinea pig and human pancreatic slices in vitro. There was a significant increase in ^{3}H-thymidine incorporation into DNA of duodenal segment of pancreas, in vivo 4 days following a single dose of NMUT (30 mg/kg; by gavage). Histological examination of pancreatic tissue up to 16 days after NMUT treatment revealed partial loss of basophilia and disappearance of zymogen granules from acinar cells, but no necrosis.

In vitro exposure of pancreatic slices to NMUT (20mM, 30 min.) resulted in a considerable enhancement of ^{3}H-thymidine incorporation into DNA. This hydroxyurea-insensitive thymidine incorporation, which is taken as a measure of DNA repair synthesis, showed NMUT dose- and exposure time-dependence. Histological examination of slices exposed to 5-20 mM NMUT revealed only a partial loss of cytoplasmic basophilia and disappearance of zymogen granules, but no necrosis.

Significance to Biomedical Research and the Program of the Institute: These studies were designed to develop an animal model to investigate the carcinogenesis of pancreatic tumors, and the underlying biochemical mechanisms of carcinogen-nucleic acid interactions. Such studies are considered to be basic to an understanding to the etiology of pancreatic cancer.

Proposed Course: The current carcinogenesis studies will be continued for the observations on the induction of tumors in pancreas and elsewhere. The pancreatic slice in vitro system will be utilized to study DNA repair synthesis following NMU and HAQO treatment, and to investigate whether DNA repair synthesis can be inhibited. Normal DNA replicative synthesis and DNA repair synthesis following carcinogen-induced DNA damage in Strain 13 and Hartley guinea pigs will be compared. DNA repair synthesis following NMU (or NMUT) and HAQO administration will be investigated in guinea pig pancreas, in vivo. Microsomal enzyme activity of the pancreas will be investigated using crude homogenates and purified microsome pellets.

Date Contract Initiated: June 29, 1972

Current Annual Level: $160,225

CHICAGO, UNIVERSITY OF (NO1-CP-43317)

Title: Definition of Sensitivity of Carcinogenesis Bioassay Systems

Contractor's Project Director: Dr. Stan D. Vesselinovitch

Project Officer (NCI): Dr. Robert A. Squire

Objectives: To establish standard bioassay systems in mice for screening substances suspected of potential carcinogenic hazard to man, making best use of the age-dependent variation in sensitivity to chemical carcinogens exhibited by F-1 hybrid mice (first generation crosses of C3HeB x A/He and C57Bl/6 x C3HeB) and to provide definition of diagnostic criteria and of detailed biologic behavior of the neoplasms produced, especially those of the liver.

Major Findings: Studies assessing the age-dependent variation in sensitivity of F-1 hybrid mice to selected carcinogens have shown a pronounced age effect in the susceptibility to carcinogenesis. Animals treated at birth or at infancy had a greater spectrum of tumor types and higher tumor incidence, than animals first treated at 42 days of age. Histogenesis and classification of renal and neurogenic tumors observed in these animals has been performed for determination of proper bioassay endpoints.

The biologic behavior of liver tumors induced by strong and weak carcinogens in infant and adult mice was explored, and indicated that all compounds tested including chlorinated hydrocarbon pesticides and aflatoxin B_1 were capable of inducing liver cancers. The nature of the lesions were confirmed by transplantation, careful examination for metastases, and lack of regression of the tumors.

Transplacental carcinogenesis studies have also shown a high degree of sensitivity in fetal tissues and tumors of the brain, kidney, RE system, stomach and ovaries have been induced.

Significance to Biomedical Research and the Program of the Institute: A major deficiency in carcinogen screening programs has been their lack of uniformity. It has been, in the past, quite impossible to estimate the relative carcinogenic potency of different compounds, even those tested in the same species, because of uncontrolled variables associated with testing protocols. These include the intrinsic differences in neoplastic response of different tissues in various strains of mice, the pronounced quantitative differences in response of fetuses, infants, and adults, and the marked effects of route of administration and dosage regimen. The dissimilarity between the histological features of murine versus human tumors of corresponding cell type has also led to disagreements, which require resolution, concerning the significance of certain neoplasms of the mouse of assessment of cancer risk in man.

The purpose of this contract is to evaluate the significance of these variables within the context of an optimal screening protocol, and to develop improved standardized procedures which will be used for future carcinogen screening programs. Definition of biologic behavior and diagnostic criteria for the histologic identification of the neoplasms that can be induced in these animals will provide needed reference standards for the evaluation of endpoints in carcinogenesis tests.

Proposed Course: Studies presently underway will be concluded in an orderly fashion and the results prepared for publication over the next 6 months.

Date Contract Initiated: June 29, 1969

Current Annual Level: $144,681

CHICAGO, UNIVERSITY OF (NO1-CP-33376)

Title: Route of Carcinogen Administration in Pancreatic Adenocarcinoma Induction

Contractor's Project Director: Dr. Stan D. Vesselinovitch

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: The objective of this project has been to explore several animal species and various routes of administration of carcinogens in an effort to

enhance the carcinogenic dose to the pancreas or the susceptibility of the target tissue. As this project nears completion, a more specific goal is completion of canine studies and an evaluation of the histogenesis of benzo[a]pyrene (BP) induced pancreatic tumors in the rat.

Major Findings: Continuation of the dosing of dogs by intraductal administration of methylnitrosourethan (MNUT) or BP was effected. Specific results are not anticipated until the animals age; however, constant observation is performed to detect any abnormalities.

Rats of both sexes were initially injected with BP at 150 and 300 mcg/rat by the intraductal route. Additional doses (1 mg/rat) were performed and animals are undergoing sacrifice and histologic evaluation of pancreas morphology during the course of the contract year.

Significance to Biomedical Research and the Program of the Institute: The need to generate biological models of pancreatic carcinogenesis has been pursued vigorously for the last three years. Significant success has been achieved in the program. This one project has pursued a distinct course. If dog carcinoma can be induced, a non-rodent species would then be available, a significant step in the achievement of the program's goal.

Proposed Course: Completion of the studies will be effected within the current contract period. Careful evaluation of the merits of this approach will be performed.

Date Contract Initiated: June 28, 1972

Current Annual Level: $104,826

CHILDREN'S HOSPITAL OF LOS ANGELES (NO1-CP-55641)

Title: Fibrinolysis as a Parameter of In Vitro Transformation

Contract's Project Director: Dr. William F. Benedict

Project Officer (NCI): Dr. Stuart Yuspa

Objective: The main objective of this contract is to determine the significance of increased fibrinolytic activity and the expression of malignancy in vivo as well as malignant transformation in vitro.

Major Findings: Cells cultured from various human and non-human malignant and normal tissues as well as mammalian cells transformed in vitro were examined for their ability to induce fibrinolysis. Generally, except for normal cells derived from lung or kidney, malignant cells had a greater ability to induce fibrinolysis than did their normal counterparts. A correlation existed between the ability of the cells to induce fibrinolysis, grow in soft agar and form tumors in immunosuppressed hosts. A notable exception was the HeLa cells which, although growing in soft agar and

forming tumors in immunosuppressed hosts, contained little fibrinolytic activity.

Significance to Biomedical Research and the Program of the Institute: These studies should help in the understanding of the relationship between increased fibrinolytic activity and the expression of malignancy in vitro and in vivo. Of equal importance is the possibility of using increased fibrinolytic activity as a marker for malignant transformation in both the fibroblast and epithelial transformation systems. It is hoped that difficulties in scoring transformed foci based only on morphological criteria can be overcome by utilizing a fibrin-agarose overlay system to quantitate the presence of transformed foci.

Proposed Course: Using the fibrin-agarose overlay technique quantitation of transformation in various in vitro systems including cells from both epithelial and non-epithelial origin will be attempted. Further studies on the levels of fibrinolytic activity in normal and malignant tissues, particularly human tumors and their normal counterparts will also be evaluated.

Date Contract Initiated: October 21, 1974

Current Annual Level: $46,818

CHILDREN'S HOSPITAL RESEARCH FOUNDATION (NO1-CP-33307)

Title: Study of the Etiology of Medulloblastoma and other Brain Tumors by a Combination of Teratogenic and Transplantable Oncogenic Treatments

Contractor's Project Director: Dr. Josef Warkany

Project Officer (NCI): Dr. Jerry M. Rice

Objectives: (1) To investigate relationship of congenital malformations and transplacental carcinogenesis and (2) to develop a model for induction of medulloblastoma and other brain tumors in rodents.

Major Findings: To test the effects of the transplacental carcinogen ethylnitrosourea (ENU) upon malformed brains, congenital cerebral defects have been produced in rats by X-irradiation of pregnant females. Among the offspring of such females micrencephaly, microphthalmia and other anomalies were common. After irradiation the pregnant rats were treated with a relatively small dose of ENU which exposed the fetuses to this oncogenic drug. The effects of this treatment on microencephalic offspring have been compared with those of normencephalic offspring treated transplacentally with ENU alone. At 15 months normencephalic offspring had a tumor incidence of 62.2% whereas those with X-ray-induced micro-encephaly had an incidence of 16.7%. The latter also showed a delay of deaths from neurogenic tumors in comparison with the normencephalic. In mice transplacental carcinogens were administered to dreher stock whose

offspring have hereditary malformations of the cerebellum. Neurogenic tumors were extremely rare in such mice as well as in "normal" offspring similarly treated.

Significance to Biomedical Research and the Program of the Institute: The results obtained so far show that under certain conditions, rats with malformations of the brain may develop fewer neurogenic tumors than normencephalic rats treated similarly with a transplacental oncogen. This finding should be considered when the long-standing theory of congenital malformations as predisposing sites of tumors is discussed.

Proposed Course: Since it is not clear why rats with X-ray induced microencephaly develop fewer neurogenic tumors than normencephalic rats experiments are under way to explore the orgins of this phenomenon. In addition attempts are being made to accelerate the appearance of neurogenic tumors to simulate childhood neoplasias.

Date Contract Initiated: March 27, 1973

Current Annual Level: $77,496

DARTMOUTH COLLEGE (NO1-CP-33378)

Title: Enhanced Delivery of Synthetic Nitroso Compounds to the Pancreas in Rats

Contractor's Project Director: Dr. Daniel S. Longnecker

Project Officer (NCI): Dr. Richard Pledger

Objectives: The principal goal is to establish an animal model for induction of pancreatic adenocarcinoma in rats using chemicals with two characteristics: (1) compounds which concentrate in the normal pancreas, and (2) compounds which appear to be potential carcinogens on the basis of chemical structure, mutagenic activity in bacteria, and/or ability to cause malignant transformation of mammalian cells in culture. To achieve these characteristics several new nitroso derivatives of amino acids and neutral red have been synthesized.

Major Findings: Several compounds have been selected for long term studies of carcinogenicity in rats on the basis of tissue localization studies, and mutagenic activity in bacterial test systems. These include azaserine, O-(N-methyl-N-nitroso-β-alanyl)-L-serine (alanoser), and puromycin. Studies with azaserine are more advanced than with other agents. During the first year, pancreas from azaserine-treated rats developed (1) nodules of atypical exocrine cells which seem to represent focal hyperplasia, and (2) encapsulated adenomas. During the second year, about one-quarter of the rats have developed adenocarcinomas of the pancreas and/or kidney. About half of the pancreatic carcinomas retain acinar cell differentiation. A few contain ductlike areas. A low incidence of atypical nodules but

no adenomas or carcinomas in pancreas were encountered among 6-20-month-old saline-treated rats. The incidence rises with age. Such nodules have been described in aged rats by others.

Alanoser is not a direct acting bacterial mutagen, but has caused in vitro transformation of hamster cells. Rats treated for 6 months with alanoser and observed for up to 14 months have developed a higher incidence of nodules of atypical acinar cells than control rats but no adenomas or carcinomas have developed. The number of atypical foci is fewer in alanoser-treated than in azaserine-treated rats. No hyperplastic or neoplastic changes have been observed in the pancreas of rats treated for 6 weeks with puromycin during 14 months of followup.

Additional compounds, e.g. methylnitrosourea derivatives of neutral red and serine have been synthesized and will be evaluated for pancreatic localization and nodule induction.

Significance to Biomedical Research and the Program of Institute: This approach has yielded a biological model of pancreatic carcinogenesis in which cancers appear to arise in acinar cells. Comparison of results with several agents should help elucidate the importance of pancreatic localization and chemical structure as determinants of pancreatic carcinogenesis by chemicals. Such information will form one basis for recognizing potential pancreatic carcinogens in the environment.

Proposed Course: Nitroso derivatives of amino acids and other carrier molecules known to concentrate in the pancreas will continue to be evaluated for mutagenic potential in bacterial systems, for transforming potential in in vitro hamster cell system, and for ability to affect the growth and differentiation of rat pancreas in vivo. Factors affecting the induction of pancreatic cancers by azaserine, e.g. age of rats during treatment, route and duration of treatment, and effect of possible co-carcinogens will be further analyzed.

Date Contract Initiated: June 28, 1972

Current Annual Level: $121,115

ERDA-NCI INTERAGENCY AGREEMENT (BROOKHAVEN NATIONAL LABORATORY) (Y01-CP-30213)

Title: Synergistic Interaction of Hormones and Neutron Radiation on Mammary Gland Carcinogenesis

Contractor's Project Director: Dr. Claire J. Shellabarger

Project Officers (NCI): Dr. Norbert Page
Dr. D. Jane Taylor

Objectives: To determine if the synergistic interaction between X-irradiation and estrogen on mammary gland carcinogenesis in the female AxC rat

holds also for neutron radiation and to understand the mechanism of the synergism.

Major Findings: To the question, "Does the synergistic interaction of X-rays and diethylstilbestrol (DES) on mammary carcinogenesis, as reported in Cancer Research 34: 166-168, 1971, in A x C rats, obtain also for neutrons?" the answer was "Yes," can be given. When 9.6 rads of 0.43 MeV neutrons were given to female A x C rats, only 2 of 35 rats developed a total of 3 mammary adenocarcinomas over a 346 day study period. When a 5 mg. pellet of DES was given, 22 of 25 rats developed a total of 182 mammary adenocarcinomas. However, when both neutrons and DES were given, 32 of 35 rats developed 842 mammary adenocarcinomas. The average latent period of mammary tumor development was shortened from 199 days in DES treated rats to 159 days in neutron and DES treated rats. Although these results with neutrons and estrogen very closely paralleled the results with X-rays and DES, they were confined to the A x C strain of rats. The interim results for an experiment in progress using the Sprague-Dawley strain indicate that DES does not induce mammary adenocarcinomas and that there is little or no interaction between DES and neutron radiation in the Sprague-Dawley strain. Another experiment, still in progress, suggests that the A x C strain shows a much larger prolactin response to DES than does the Sprague-Dawley strain and thus prolactin is implicated in the synergistic mammary adenocarcinoma response to neutrons and DES noted in the A x C strain.

Significance to Biomedical Research and the Program of the Institute: It has been reported that X-radiation and DES interact in a synergistic manner in the induction of mammary adenocarcinoma in female rats of the A x C strain. Other studies have indicated a large relative biological effectiveness (RBE) for neutron-irradiation in the induction of mammary neoplasia in female rats of the Sprague-Dawley strain. Because it has been reported that women exposed to repeated fluoroscopic examinations, women survivors of atomic bombings, and possible women treated with X-ray for acute postpartum mastitis, all have an increased risk of developing breast cancer, radiation exposure is now associated with breast cancer of the human female. With more frequent use of neutron services in industry, research, and medicine, the possibility of increased exposure of women to neutron-irradiation is expanded. Also, the use of steroids for contraceptive and/or replacement therapy has increased. Thus, the interactions of neutron-irradiation and sex steroids in regard to mammary carcinogenesis should be evaluated. This project is an attempt to develop an animal model,and to understand the model itself, so that the qualitative aspects of mammary carcinogenesis in man may be evaluated in terms of cause and prevention of breast cancer.

Proposed Course: The synergistic response to neutrons and DES on mammary adenocarcinoma formation noted in the A x C female rat will be tested at lower doses of neutron radiation and in regard to the role that prolactin may play in the synergistic response.

Date Contract Initiated: June 28, 1973

Current Annual Level: $90,000

HAZLETON LABORATORIES, INC. (NO1-CP-23287)

Title: Induction of Adenocarcinoma of the Prostate

Contractor's Project Director: Dr. Daniel W. Dalgard

Project Officer (NCI): Dr. Richard A. Pledger

Objective: The objective is to attempt the induciton of adenocarcinomas of the canine prostate by a variety of hormonal stimuli and carcinogen treatments. Animals from different age groups are used in this study.

Major Findings: A high incidence of fibrosarcomas were induced in the immature age group of dogs using 3-methylcholanthrene. However, no adenocarcinomas or other forms of neoplasia have been detected to date.

Significance to Biomedical Research and the Program of the Institute: Deaths due to prostatic adenocarcinoma appraoch 20,000 in the U.S. annually. Unfortunately, there are no known animal species which exhibit a truly comparable disease entity. The dog, however, does develop age associated prostatic hyperplasia which is quite similar to that seen in man. Morphologically, the gland is more similar than that of the rodent and most other conventional laboratory species.

Proposed Course: Sixty male dogs 5-years-old or older are receiving direct intraprostatic inoculations of methyl nitrosourea (MNU) at weekly or biweekly intervals. Palpation and perineal punch biopsies are performed at six month intervals. The design also includes the use of castrated dogs, intact dogs, and dogs which receive supplementary androgens.

Date Contract Initiated: June 24, 1972

Current Annual Level: $72,264

KANSAS, UNIVERSITY OF (NO1-CP-23271)

Title: Histogenesis of Guinea Pig Pancreatic Adenocarcinoma

Contractor's Project Director: Dr. Janardan K. Reddy

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: This project was initiated to confirm earlier reports of induction of pancreatic adenocarcinoma in guinea pigs by N-methyl-N-nitrosourea (MNU) and to study the histogenesis of pancreatic tumors. An

additional objective of this project is to manipulate this model so as to reduce the induction period and increase the incidence of pancreatic adenocarcinoma.

Major Findings: Pancreatic adenocarcinomas developed in 10 of 34 inbred strain 13 guinea pigs between 28-44 weeks of MNU treatment. In other animals atypical changes involving acinar cells were observed in some pancreatic lobules. These changes consisted of ductular or pseudoductular transformation, acinar ectasia, increased mitotic activity and periacinar fibrosis. These changes are suggestive of acinar cell dedifferentiation and their role, if any, in the histogenesis of pancreatic adenocarcinoma remains to be elucidated. These studies suggest the feasibility of using inbred guinea pigs for developing a satisfactory model of pancreatic adenocarcinoma. Additional studies are necessary to minimize the high mortality rate (54%) during the first six months and to enhance the incidence of pancreatic adenocarcinoma.

By using a single intravenous injection of 4-hydroxyaminoquinoline-1-oxide, a model to induce pancreatic necrosis and regeneration in strain 13 inbred guinea pigs was developed. This model of pancreatic regeneration should be useful to study the potentiating effect of MNU on the incidence of pancreatic tumors when administered during peak regeneration.

Significance to Biomedical Research and the Program of the Institute: The incidence of pancreatic adenocarcinoma in this country is progressively increasing and there is as yet no reliable animal model of this tumor. The development of a biological model of pancreatic carcinoma is most important to the program. Until such time that a reproducible, experimental protocol is available which characteristically induces cancer of the pancreas, the research necessary to enable an understanding of the cause and prevention of this disease will be markedly restricted. Model development is one of the major goals and this project along with others is designed specifically to achieve this goal.

Proposed Course: Additional studies, using large numbers of inbred strain 13 guinea pigs will be undertaken to confirm the rapid development of pancreatic tumors in this inbred strain. Sequential morphologic studies to determine the histogenesis, particularly the role of acinar cell dedifferentiation, and progression of this cancer will be continued. Attempts to increase the incidence of tumors will be made using various pancreaticotoxic agents such as ethanol, 4-hydroxy-aminoquinoline-1-oxide and di-isopropanolnitrosamine. The long-term effects of di-isopropanol-nitrosamine on the pancreas of strain 13 guinea pigs will also be studied.

Date Contract Initiated: June 10, 1972

Current Annual Level: $46,872

LITTON BIONETICS, INC. (NO1-CP-22063)

Title: Resource for Preparation and Examination of Experimental Biological Material for Histological and Ultrastructural Study

Contractor's Project Director: Dr. Elizabeth W. Kingsbury

Project Officer (NCI): Dr. Curtis Harris

Objectives: The main objectives of this program are the preparation and examination of tissues for light and electron microscopy to provide information about the morphology, mitotic activity, and metabolic activity of organs and cell systems as they relate to carcinogenesis. Primarily, this work will aid in the development of the rat prostate as an organ model for carcinogenesis studies. Other tissues to be studied are rat mammary cells, hamster trachea and bronchi, and mouse epidermal cells. Additionally, various support projects such as autoradiography studies are undertaken at the discretion of the Project Officer.

Major Findings: Light and electron microscopic evidence demonstrates the arrangement and interrelationships of the various lobes of the prostatic complex and that the lobes and accessory organs are clearly identifiable from one another. As a result, investigators will be able to identify any prostatic tissues removed for study. Cultured explants of ventral and dorsal prostatic tissue have been treated with various hormones to study possible effects on growth rate and to establish baselines for further carcinogenesis studies. Because spermine and spermidine have been noted in prostatic fluids, organ cultures have been treated with testosterone, spermine, spermidine, and propylene glycol to determine any ultrastructural alterations. Autoradiographic (ARG) and ultrastructural investigations are in progress.

The following projects have been completed: (1) The in situ embedded rat mammary epithelium cell cultures which had been treated with various hormone combinations were examined to establish morphology and cell growth parameters for further study. There were no significant differences between groups and it was determined that future E.M. studies should be based on experimental evidence derived from other sources such as biochemistry or light microscopy. (2) Because mouse epidermal cells respond to phorbol ester treatements by marked growth stimulation which is altered by DMBA, a project was designed to investigate ultrastructural changes which may indicate malignant transformations. First results indicate definite nucleolar alterations due to treatment and experiments are continuing in order to confirm this. (3) A study is in progress to determine the ultrastructural changes in tracheal and bronchial epithelium during influenza virus induced epithelial hyperplasia. Thick sections of hamster tissue have been evaluated and blocks have been selected for electron microscopic examination. A significant number of micrographs has been produced and work is nearly completed which will add to other data on susceptibility states and chemical carcinogenesis. (4) Eighteen service projects were received which required thick sectioning for ARG or light

microscopy or projects which required counting and evaluation of autoradiographic slides.

Significance to Biomedical Research and the Program of the Institute: As the incidence of prostatic cancer is frequent in humans, it is necessary to establish an animal model system to study the mechanisms of prostatic carcinogenesis. Additionally, it has become desirable to study the effects of the increasing levels of carcinogens in the environment, so that their modes of action can be defined and protection devised.

Proposed Course: The effects of various carcinogens and hormones on organ cultures of the prostate will be studied. The secretory and other granules found in earlier studies will be examined histochemically to help establish functional differences between lobes. Studies of the effects of phorbol ester and DMBA on mouse epidermal cells will continue as will the viral hyperplasia study. Other short term projects will be completed as requested by NCI.

Date Contract Initiated: December 1, 1971

Current Annual Level: $133,141

MARYLAND, UNIVERSITY OF (NO1-CP-23206)

Title: Studies on the Histogenesis of Renal Carcinoma

Contractor's Project Director: Dr. Benjamin F. Trump

Project Officer (NCI): Dr. Richard Pledger

Objectives: The objective of this contract is to develop a model for renal carcinoma in experimental animals. The present model uses a carcinogen, N-4-4' fluorobiphenylacetamide (FBA), administered in the diet to rats. The focus of the study is the definition of the progression of changes in the tubule from normal to malignant lesions and to correlate each stage with similar lesions occurring in the human kidney.

Major Findings: Groups of male F344 rats fed a diet containing 0.04% FBA developed large tumors in the kidney cortex in 2/3 of the animals by approximately one year. The tumors were frequently multiple and varied in size from pinpoint to two centimeters in diameter. The larger tumors often showed necrosis in the center of the mass. All tumors appeared to originate within the cortex, although in the case of the large tumors, they occupied most of the renal mass.

The tumors all appeared to be epitelial and both solid and papillary patterns were seen. Sarcomas have not been identified. The tumor cells were cuboidal to columnar, often showed specializations of the cell membrane resembling the brush border of proximal tubules and showed basement membrane material both at the base and between the cells in

large tumor masses. Electron microscopic characteristics resembled those published for the human.

With respect to histogenesis, the earliest hyperplasias were seen by 12 weeks and consisted of tubular forms having small crowded, basophilic cells with small, dense nuclei. Occasional cells with giant nuclei were also seen. By 24 weeks larger hyperplastic nodules often appeared to originate from growth of cells into the lumens of tubules. Again, these cells had a well developed brush border and a terminal web-like area just beneath the plasma membrane. By 36 weeks multiple nodules of hyperplasia were identified. These nodules became larger and began to resemble the cells in the large tumors seen by 1 year. Isologous transplantation studies reveal that even very small lesions (less than 2 mm in diameter) can be transplanted successfully thus indicating that the small lesions are malignant. Isologous intrarenal implantation of tumor wedges into young, healthy rats resulted in large renal tumors which showed metastasis to lung.

Histochemically, the tumors showed a marked depression of glucose-6-phosphatase activity and succinate dehydrogenase activity while the activities of glucose-6-phosphate dehydrogenase, lactate dehydrogenase and alpha-glycerophosphate dehydrogenase were similarly enhanced compared to the strongest reacting components of the normal nephron.

During the past year attempts were made to shorten the long time required for tumor development by addition of 1% lead acetate to the FBA diet mixture. It was thought that the mitogenic effect of lead would aid tumor development in a co-carcinogenic manner. In comparison with similar length exposure to FBA alone the effect of administration of carcinogen and lead was to produce more tumors, larger tumors and to decrease the time necessary for large tumor development.

In addition, the tumors produced by feeding carcinogen alone for 6 months and then lead plus carcinogen for an additional 6 months were extremely large and in one animal metastasis to pancreas, broad ligament, and medial musculature of the thigh had occurred. It is felt that the increased incidence of tumor and shortened time necessary for tumor production which characterize concurrent lead and fluorobiphenyl acetamide exposure need to be further studied in an attempt at understanding possible synergistic effects of environmental heavy metals on carcinogenesis.

Studies of 800 cases of human renal carcinoma revealed a percentage incidence of 17-18% of multiple small lesions away from the main tumor mass and similar to those seen in the rat. These ranged from focal changes involving a few cells in a proximal tubule to small areas of hyperplasia. So far the human lesions resemble those seen in the rat model in morphology, histogenesis, and pattern of growth in culture. Electron microscopic studies to date on the human also show morphology compatible with that seen in the rat, although many further studies are presently underway.

Significance to Biomedical Research and the Program of the Institute: These studies are mainly of significance in that they appear to be promising with respect to development of an animal model of human renal carcinoma. This would obviously be of great value in terms of studying carcinogenic effects, including the effects of environmental agents, management, and even newer methods of diagnosis.

The development of the model may also provide another excellent model with which to study the mechanism for carcinogenesis because of the wealth of information about the structure and metabolism of the kidney.

Proposed Course: The efforts during the coming year will be concentrated on completing electron microscopic and histochemical studies of human renal carcinomas to compare with the rat model. In addition, emphasis will be placed on the study of the clear cell in which factors related to the formation of these cells will be elucidated.

Date Contract Initiated: February 1, 1972

Current Annual Level: $91,957

MASON RESEARCH INSTITUTE (NO1-CP-23238)

Title: Study of the Uptake and Excretion of Carcinogens In and Their Effect on the Prostate

Contractor's Project Director: Dr. Emil R. Smith

Project Officer (NCI): Dr. Richard Pledger

Objectives: The uptake of carcinogenic chemicals by the prostate and their secretion in prostatic fluid is being studied in the rat and dog in order to determine in some detail what factors govern the ability of chemicals to penetrate into the prostate and its secretion.

Major Findings: Following the systemic administration of known carcinogenic chemicals to rats, the relative tendency to accumulate in the dorsolateral lobes, which were combined for analysis, and in the ventral lobe was similar; the rank order for maximum tissue to plasma ratios was 7,12-dimethylbenz(a)anthracene (DMBA)>3-methyl-cholanthrene (MCA)>3-amino-1,2,4-triazole (ATA)>aflatoxin B_1 (AFLA) and 2-acetylaminofluorine (2-AAF). Ratios for cadmium (Cd) varied widely and could not be interpreted in this manner. In dogs with prostatic fistulas, the relative penetrability into resting prostatic fluid as reflected in prostatic fluid to plasma ratios was DMBA>MCA>2-AAF>AFLA>ATA>Cd. When prostatic secretion was stimulated by pilocarpine, the concentration of DMBA and MCA in prostatic fluid fell, while that of ATA rose and that of AFLA was unchanged. Thus the penetration of carcinogenic chemicals into the prostate and its secretion was a function of the plasma level of the chemical, the physical-chemical properties of the compound, the lobe of the gland and its secretory state.

Significance to Biomedical Research and the Program of the Institute: The present experiments, when complete, will provide a rationale basis for attempts to produce an animal model of prostatic adenocarcinoma and will produce some insight into the possible role of chemicals in the etiology of human prostatic adenocarcinoma.

Proposed Course: The studies conducted concern primarily the uptake of carcinogenic chemicals by the prostate of the rat and their secretion by the prostate of the dog. The experiments to be conducted during the next period of the contract include uptake by the dog prostate and secretion by the rat prostate.

Date Contract Initiated: June 15, 1972

Current Annual Level: $114,466

MELOY LABORATORIES, INC. (NO1-CP-55613)

Title: Transplacental Carcinogenesis in Erythrocebus Patas

Contractor's Project Director: Dr. D. Lewis Sly

Project Officer (NCI): Dr. Jerry Rice

Objectives: This project is designed to provide a non-human primate model which will demonstrate transplacental chemical carcinogenesis.

Major Findings: As this is a new contract, no major finding can be reported at this time.

Significance to Biomedical Research and the Program of the Institute: With the recognition of the association between exposure to diethylstilbestrol in utero and development of vaginal adenocarcinoma during the second decade of life, the possible significance to human health of transplacental chemical carcinogens has become increasingly a matter of concern. Experimental studies on transplacental chemical carcinogenesis have been limited to rodent species, which differ significantly from man in many ways likely to be of importance for understanding the different consequences of exposure to chemical carcinogens during fetal versus adult life. Among these are the much more rapid rates of fetal and neonatal development and maturation in rodents.

Proposed Course: With the establishment of a breeding colony of Erythocebus patas, 1 ethyl-1-nitrosourea, a potent carcinogen, will be used to demonstrate transplacental chemical carcinogenesis. Particular emphasis will be placed on defining the periods of maximal sensitivity in different organ systems and demonstrating parallels between both the morphological and clinical behavior of corresponding human tumors, and the results obtained in rodents with the same carcinogen.

Date Contract Initiated: October 23, 1974

Current Annual Level: $146,331

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CP-43232)

Title: Identification of Site of Origin of Human Pancreatic Adenocarcinoma

Contractor's Project Director: Dr. Patrick J. Fitzgerald

Project Officer, (NCI): Dr. Richard Pledger

Objectives: To identify the cell origin of adenocarcinoma of the pancreas using light microscopic, histochemical, and electronmicroscopic techniques; to determine the route by which pancreatic carcinoma invades and metastasizes; to study associated hyperplastic, metaplastic, and carcinoma in situ lesions in patients with pancreatic carcinomas; and to classify and clarify the nomenclature of nonendocrine pancreas carcinomas.

Major Findings: Retrospective Study: (1) The study of histologic sections of 381 cases (115 autopsies, 99 major surgical specimens, and 167 biopsies) of nonendocrine pancreas carcinoma indicated that morphologic patterns of these tumors could be delineated as follows: Duct cell adenocarcinoma (76%), Giant cell carcinoma (5%), Microadenocarcinoma (4%), Adenosquamous carcinoma (3%), Mucinous adenocarcinoma (2%), Anaplastic carcinoma (2%), Cystadenocarcinoma (1%), Acinar cell carcinoma (1%), Carcinoma in childhood (under 1%), and Unclassified carcinoma (7%).

(2) In 195 cases of patients with pancreas carcinoma, search was made for changes in the pancreas duct epithelium in a control group of 100 pancreases from autopsies of patients with non-pancreas cancer. The following incidences were found for pancreas cancer and non-pancreas cancer, respectively: Mucous cell hypertrophy, 39% versus 28%; pyloric gland metaplasia, 42% and 12%; a typical duct hyperplasia, 14% and none; carcinoma in situ in 19% and none in the control group.

Prospective Study: Light Microscopy: Twenty-three new cases involving the pancreatoduodenal region have been studied. The histologic diagnoses were: duct adenocarcinoma (12 cases); ampullary carcinoma (5 cases); islet cell carcinoma (2 cases); duodenal carcinoma (2 cases); adenosquamous carcinoma (1 case); giant cell carcinoma (1 case).

In 3 of the duct adenocarcinomas, in situ changes were present in large duct adjacent to the invasive tumor.

Significance to Biomedical Research and the Program of the Institute: It is possible that specific morphologic groups can be related to specific etiologic or carcinogenic factors. One of the adenosquamous cancers had a history of multiple tumors occurring in the field of irradiation to the retroperitoneum (skin cancer, renal pelvis cancer and pancreatic cancer).

Chemotherapeutic compounds may have differential effects on the cancer type of subtype, just as ethionine has been shown to affect the normal acinar cell more than it does the normal duct, or as streptozotocin has been demonstrated to cause more damage to the beta islet cell than it does to other cell types.

Different subtypes may have different prognosis, as it is known for the cystadenocarcinoma or islet cell carcinoma compared with the most common duct carcinomas.

Although the significance of hyperplastic and metaplastic changes in pancreatic ducts is uncertain, at this moment, it would appear that the 19% incidence of in situ carcinoma would suggest that there may be a significant latent period between the appearance of in situ carcinoma and the grossly evident cancer, analogous to the long interval between in situ and invasive carcinoma of the cervix.

Proposed Course: To attempt to correlate different histologic types with specific anatomic sites of the normal pancreatic duct using histochemical techniques e.g.: mucin in tumors forming large glands at the head of the pancreas to be compared with the amount of mucin, if any, in small ducts; to continue the survey of pancreas cancers as to ultrastructural details in order to determine if the subtypes found by light microscopy have any common features when viewed by the electron microscope, e.g.: is the giant cell tumor merely a more rapidly growing adenocarcinoma? Is the anaplastic tumor arising from a duct, acinar, or islet cell?; to study carcinomas of the ampulla or duodenum to determine if there are differences from the pancreatic duct cancers; and to correlate associated changes in the duct with clinical epidemiologic data such as smoking, occupation, etc.

Date Contract Initiated: June 1, 1974

Current Annual Level: $33,443

MICHIGAN, UNIVERSITY OF (NO1-CP-33331)

Title: Organ Culture of the Prostate: A Biological Model

Contractor's Project Director: Dr. Raymond H. Kahn

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: To determine the requirements, both physical and chemical, for maintaining normal dog and human prostate glands outside the body as organized tissue (organ culture) and to establish the structural criteria for normal prostatic tissue.

Major Findings: A technique has been developed which permits the maintenance of explants of dog prostate in organ culture for 9 to 18 days. Some of the conditions which have been shown to be important for maintenance include

the nutrient (Trowell's T-8), the gaseous environment (50% oxygen) and the male steroid hormone Testosterone. The latter has been shown to have rather specific dose requirements (i.e. $5x10^{-6}$M). Attempts to further reduce tissue damage at the cellular level by the addition of hydrocortisone and similar adrenal steroids caused increased viability, but also produced undesirable metaplasia as well. The limited availability of sufficient viable human prostatic tissue has impeded progress in this species.

Significance to Biomedical Research and the Program of the Institute: Although prostatic cancer is one of the leading causes of death in elderly men, there is no available model systems for studying the normal prostate nor the natural history of change with age. While the dog seems to be the most closely related model, investigations using the intact animal are both expensive and time consuming. Moreover, in the intact animal, it is also difficult to distinguish changes in the prostate due to experimental regimen from those due to organ interactions, serum hormone levels, etc. The development of a biological model maintained in organ culture would permit the replication of the body's response under carefully controlled conditions, divorced from organ interactions. Such a model would lend itself to an assessment of the cause of prostate cancerous changes (carcinogenesis) and evaluation of treatment protocols. Once a model system has been established, early diagnosis of lesions also would be conceivable.

Proposed Course: Further criteria for maintaining the dog and human prostate in organ culture are currently being investigated. Included in this investigation are the role of Vitamin A, the pituitary hormone Prolactin, Polyamines which appear to be characteristic of the prostate gland, as well as the effect of pH.

Date Contract Initiated: June 25, 1973

Current Annual Level: $114,035

MICHIGAN, UNIVERSITY OF (NO1-CP-45619)

Title: Purification of Epidermal Chalone, A Tissue Specific Inhibitor of Cellular Proliferation

Contractor's Project Director: Dr. John J. Voorhees

Project Officer (NCI): Dr. Henry Hennings

Objectives: Chalones have been defined as tissue specific, species nonspecific inhibitors of cell proliferation. The isolation and purification of such an inhibitor which exerts its effects in the G_1 phase of the cell cycle is the primary objective of this contract.

Major Findings: Basal and spinous cells are obtained from newborn rat epidermis by trypsinization. The cells are placed in tissue culture medium containing ^{3}H-labeled mannose and fucose for regeneration of the cell surface.

Portions of the regenerated cell surface are obtained by sonication, trypsinization, pronase and neuraminidase treatments. After chromatography on Bio-Gel P-30 columns, six different peaks have been isolated. The 2nd and 4th peaks inhibit both the epidermal cells and fibroblasts with a certain amount of problems encountered with respect to tritiated thymidine (TdR) metabolism. However Peak III appears to contain a potent inhibitor (79%) of epidermal cell proliferation as determined by a terminal 3 hour pulse label of ^{3}H-TdR. Fibroblast proliferation and TdR metabolism seem to be unaltered by this fraction. Peak III contains radioactive mannose and fucose in addition to a protein component.

Significance to Biomedical Research and the Program of the Institute: The studying of any system which controls the rate of cell proliferation has possible implications for the control of malignant growth. If tissue specificity is a component of the system, it should be of even greater value since influences on normal systems would be minimal.

Proposed Course: A substantial quantity of Peak III will be prepared and additional purification procedures (DEAE or CM-cellulose column chromatography) will be attempted. This fraction will also be tested in vivo.

Date Contract Initiated: June 1, 1974

Current Annual Level: $102,275

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP-02199)

Title: Laboratory Service for Support in Carcinogenesis Bioassay and Related Activities

Contractor's Project Director: Dr. Russell M. Madison

Project Officer (NCI): Dr. Jerry M. Rice

Objectives: This contract provides scientific collaboration to the principal areas of investigation within the Carcinogenesis Program of the National Cancer Institute. Facilities are available for in vivo experimentation as well as in vitro studies utilizing cell culture and biochemical methods. The contract operates histology laboratories which prepare paraffin-embedded sections for light microscopy, and epoxy-embedded sections for electron microscopy or high resolution light microscopy. This varied pool of technological ability is employed to develop and utilize animal models for the major forms of human tumors including tumors of the lung, pancreas, prostate, bladder, mammary gland, and gastrointestinal tract. Facilities utilizing cell and tissue culture are employed for the analysis of the carcinogenic process.

Major Findings: Major areas of experimentation have included the following: (1) Tests to develop new animal models for (a) Pancreatic carcinoma in guinea pigs; (b) Prostatic carcinoma in rats and (c) Gastrointestinal

carcinoma in rats; (2) efforts to utilize existing animal models for the characterization of the carcinogenic process, and evaluation of possible corrective measures, including (a) modifying influences of dietary factors on the induction of mammary tumors, liver tumors, bladder tumors, neurogenic tumors, kidney tumors, and gastrointestinal tumors; (b) modifying influences of environmental factors on the induction of tumors, including co-carcinogens and synergistic carcinogen effects; (c) enhancement of normal immunological responses against induced tumors of the respiratory system; (d) development of in vitro techniques for identifying human populations at risk of induction of respiratory tumors; and (e) development of in vitro screening tests for identifying potential carcinogens.

Significance to Biomedical Research and the Program of the Institute: This contract offers the ability to conduct long-term experiments involving large numbers of experimental animals, to make rapid tests of new approaches in experimental pathology related to chemical carcinogenesis in specific organ systems, and the ability to follow up rapidly on studies that demonstrate relevance to the overall objectives of the National Cancer Institute.

Proposed Course: During the course of the last year, this contract has moved into an enlarged facility. This contract will continue to offer a diversified facility for support of, and collaboration with NCI staff scientists in the Carcinogenesis Program. The increase in capacity will add to the volume of experimentation conducted. Emphasis within the Carcinogenesis Program is expected to be diversified and changing in nature, and it is our ultimate objective to react to such a program with minimum delays and maximum quality of effort.

Date Contract Initiated: June 4, 1970.

Current Annual Level: $881,474

NEBRASKA, UNIVERSITY OF (AT LINCOLN) (NO1-CP-33289)

Title: Chemical Carcinogen-Induced Noduligenesis and Tumorigenesis in Whole Mouse Mammary Gland Organ Culture

Contractor's Project Director: Dr. Mihir R. Banerjee

Project Officer (NCI): Dr. Richard R. Bates

Objectives: To determine whether preneoplastic (nodule) or neoplastic "transformation" of mouse mammary epithelium can be induced by chemical carcinogens during hormone induced development of the mammary gland in organ culture.

Major Findings: The entire mammary gland of the immature mouse can be induced to pregnancy-like lobulo-alveolar growth in organ culture medium supplemented with insulin + prolactin + estradiol + progesterone + aldosterone. Cultivation of the alveolar gland in medium with the lactogenic hormones,

insulin + prolactin + cortisol, induces milk-like secretory material. The milk-like material has been determined to contain casein by radioimmunoassay. Subsequent incubation of the gland in hormone deficient medium (with insulin alone) results in regression of the alveoli, and the gland resembles the post-lactational involuted parenchyma. Thus, the entire biological cycle of the mouse cells is reproducible in organ culture of the whole mammary gland.

Treatment of the mammary cells with the carcinogenic hydrocarbons, 3-methylcholanthrene (MCA) or 7,12, dimethylbenzathracene (DMBA), during the initial growth phase in organ culture induces hyperplastic alveolar nodules in a large number of glands. No similar hyperplastic lesions are present in nontreated control glands. Induction of these nodules is highly reproducible and mammary gland of MTV-free C3Hf and BALB/c mice so far studied are susceptible to chemical carcinogen-induced noduligenesis in organ culture. The lesions are detectable in the regressed gland after 10-14 days cultivation in vitro. Histologically, the nodules resemble the precancerous mammary nodules induced in vivo by the mouse mammary tumor virus (MuMTV). After transplantation in the gland-free mammary fat-pad in vivo, some of these lesions produce alveolar outgrowth, a feature also characteristic of the precancerous mouse mammary nodule in vivo. Thus, hyperplastic alveolar nodules, morphologically and histologically similar to the precancerous mammary nodules, are inducible by chemical carcinogens, exclusively in organ culture of the whole mammary gland.

Significance to Biomedical Research and the Program of the Institute: The Biological Models Segment has the goal of developing appropriate animal model for studying the biology and pathogenesis of major forms of human cancer. Cell and organ culture systems provide an effective tool for these studies without involving the complexities of the entire animal. The unique organ culture procedure utilized in this project permits hormonal induction of development of the entire breast in the culture medium. The results presented above, so far, strongly indicate that hyperplastic alveolar nodules (similar to the preneoplastic mammary nodules in vivo) can be induced by chemical carcinogens in the mammary gland within 2-3 weeks in organ culture (in animal the same process may take 6 months to a year). These observations are considered very encouraging. Consistent with the objective of the Biological Models Segment and the National Cancer Institute, development of an in vitro carcinogenesis system in organ culture of whole mammary gland will provide an important tool for studying the pathology of hormone-carcinogen interactions in breast cancer and also a rapid relatively less expensive assay system for testing various environmental carcinogens exclusively in the petri dish.

Proposed Course: Emphasis now is to ascertain the tumor potential of the chemically-induced mammary nodules in organ culture. The following sets of experiments are in progress: (1) transplantation of the nodules in gland-free mammary fat-pad of young virgin female; (2) fat-pad transplantation of the nodules into virgin female hormonally stimulated by pituitary isograft; (3) fat-pad transplantation of nodules in hosts prestimulated by pituitary grafting; (4) fat-pad transplantation of dissociated

epithelial cells of carcinogen treated gland in young virgin hosts with or without hormonal stimulation.

These animals are terminated from time to time for morphologic examination of the characteristics of the outgrowth produced by the explant.

Date Contract Initiated: April 1, 1972

Current Annual Level: $67,000

ORGANIZATION FOR HEALTH RESEARCH (TNO) (NO1-CP-33330)

Title: Synergistic Interaction of Hormones and Neutron Radiation on Mammary Gland Carcinogenesis

Contractor's Project Director: Dr. J. J. Broerse

Project Officers (NCI): Dr. Norbert Page
Dr. Richard Pledger

Objectives: To estimate relative biological effectiveness (RBE) values for three different neutron energies in three rat strains of expected different susceptibility to induction of mammary tumors by ionizing radiation. To analyze causes of differences in susceptibility between rat strains and to study the modifying influences of estrogen administration.

Major Findings: For all three neutron energies the dosimetric measurements have been completed and the irradiation conditions for the neutrons and the X-rays have been designed to arrive at directly comparable exposure conditions. A breeding colony of Sprague-Dawley (SD) rats has been started and the production of this strain as well as the Brown Norway (BN) and the Wistar (WAG) is meeting the requirements of the study. Nearly all groups of intact as well as sterilized females have been exposed to the designed x- and neutron- irradiation, totaling over 2,000 animals, and the first tumors have appeared. Studies are underway to determine the optimal method of administraiton of E-2 (Estradiol-17 beta) to provide a continuously increased hormone level of three to five times the normal value. The determinations of both the cellular and humoral immune responses of the three rat strains at various ages have been initiated.

Significance to Biomedical Research and the Program of the Institute: The Program is expected to provide an insight into the interaction of estrogen levels and radiation carcinogenesis in the mammary gland which is of relevance to the problem of human breast cancer.

Proposed Course: Introduction of the hormone treated irradiated groups. With regards to the combined action of estrogen and radiation, emphasis is placed on the monitoring of plasma hormone levels. For this reason, E-2 remains the stimulator of preference but adequate additional groups treated with DES will be introduced to allow comparison of our results with those of the other contractors. After identification of different patterns of

tumor incidence in the main groups, determinations of antigenicity of tumors, estrogen binding capacity of tumors, and of plasma levels of prolactin will be performed for appropriate samples of the experimental rat population.

Date Contract Initiated: June 25, 1973

Current Annual Level: $86,433

PORTLAND STATE UNIVERSITY (NO1-CP-43256)

Title: Isolation and Maintenance of Normal Human Prostatic Epithelial Cells Lines

Contractor's Project Director: Dr. Thelma N. Fisher

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: The purpose of this project is to isolate, propagate, establish nutritional requirements and to devise methods of measuring normal human prostatic epithelial growth in tissue culture. Such systems will be useful for in vitro investigations of malignant conversions induced by chemical carcinogens.

Major Findings: Suitable conditions for the routine propagation and maintenance of cellular outgrowth as achieved with human prostatic explants are proposed. Most consistent results are obtained when explants are cultivated initially in Eagle's medium containing 30% fetal bovine serum and when subsequent feedings are made with fluid which include 20% fetal bovine serum. Other important constituents of growth fluids are putrescine, insulin or testosterone, essential and non-essential amino acids, glutamine, vitamins, penicillin and streptomycin. Double strength quantities of amino acids, glutamine and vitamins are added as cell numbers increase. Antifibroblast antiserum in the presence of complement is used to destroy contaminating cells displaying typical fibroblast morphology. If cell sheets appear to constrict as duplication at the periphery of the clone diminishes, cultures are treated lightly with trypsin to relieve this surface phenomenon so that duplication can continue.

Storage studies are underway. In early experiments human prostatic tissues were stored at liquid nitrogen temperatures in solutions containing Eagle's medium fortified with 2% fetal bovine serum and 10% DMSO or in such a mixture to which glycerol was added. Some outgrowth was achieved when explants were replated after short storage intervals in these solutions. Presently, explant sections are held in Eagle's medium containing 20% fetal bovine serum, single strength media constituents as mentioned above but without hormones, plus 10% DMSO. Samples are removed at weekly intervals. Preparations are washed free of DMSO and replated. To date growth has been achieved from 10 explants retained up to 3 weeks in liquid nitrogen. However, thus far, tissues from only 7 patients have been tested for a maximum of 3 weeks storage. Autoradiographic procedures using ^{3}H-thymidine to

measure DNA incorporation, replication etc. are in progress. Methods for the above, including clearing of background tritium from autoradiograms are successful in time studies with H.Ep.2 cell preparations.

Significance to Biomedical Research and the Program of the Institute: It has been difficult for investigators to obtain sufficient quantities of normal human prostatic epithelium in order to conduct measurements of malignant conversion as induced by chemical carcinogens. With a controlled and adequate in vitro system available, it should be possible to document initial events which occur in such transformations.

Proposed Course: To continue storage studies as new tissues become available. It is important to document epithelial proliferation as evidenced by ^{3}H-thymidine incorporation into DNA, coincident with the presence of mitotic figures and to show actual linear increase of cell numbers on plastic petri dishes scored in 2mm squared units. Using these methods it should be possible to note the effects of testosterone, insulin, prolactin, putrescine, double and single strength amino acids and other components on growth.

Date Contract Initiated: December 28, 1973

Current Annual Level: $50,000

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CP-33379)

Title: Genadal Hormonal Effects on Prostate

Contractor's Project Director: Dr. Sydney A. Shain

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: The role of the changing endocrine status of the prostate in the pathogenesis of prostatic carcinoma is ill-defined and poorly understood. With the view that prostatic carcinoma represents aging-associated dystrophic alterations in the regulation of cellular homeostasis, two specific components of prostatic cellular metabolism either known or thought to be intimately involved in the regulation of prostatic epithelial cell function will be evaluated. These components of cellular metabolism are the cellular content,specificity, and distribution of prostatic androgen receptors and prostatic androgen metabolism. The objective of this contract is to evaluate aging-associated changes in these aspects of cellular metabolism in the rodent, canine, and human prostate, and (2) to identify changes in these specific aspects of prostatic metabolism which could result in alterations in the regulation of cellular homeostasis and to define the comparative endocrinology of the prostate of the three species in order to provide guidelines for the development of a model for human prostatic carcinogenesis.

Major Findings: A mince saturation analysis protocol has been developed for the simultaneous analysis of total cytoplasmic and nuclear androgen

receptor sites in rodent prostate. Employing this protocol total androgen receptor content and distribution in prostate of aging rodents has been evaluated. As Sprague-Dawley animals age from 105 to 610 days, the mince saturation protocol reveals a decrease of 40 percent in the level of ventral prostate cytoplasmic androgen receptors. Application of this protocol to ventral prostate tissue from A x C rats revealed a decrease in the cytoplasmic androgen receptor content of 70 percent as the animals aged from 137 to 976 days. Identical analyses performed on dorsolateral prostate tissues from the above animals failed to provide any evidence for a cytoplasmic androgen receptor. Saturation analysis of the nuclear androgen receptor content of these tissues revealed a previously unreported aging-associated reduction in the per-cell receptor site content. The above described Sprague-Dawley animals evidenced in highly significant aging-associated decrease of 25 and 50 percent, respectively, in the nuclear receptor content of the ventral and dorsolateral prostate. Similarly, A x C animals evidenced a decrease of 40 to 50 percent, respectively, in the nuclear receptor content of the ventral and dorsolateral prostate. A modification of the protocol utilized in the preceding studies has been applied to tissues from intact animals. This study revealed a reduction of 65 and 25 percent, respectively, in the cytoplasmic and nuclear androgen receptor content of the ventral prostate of A x C animals respectively aged 290 and 1040 days. Quantitative identity of the decrease in prostatic androgen receptor content for the intact and castrated animals establishes the validity of the observations.

Aging in the rat is associated with marked changes in the androgen metabolic potential of both the ventral and dorsolateral prostatic complexes. Ventral prostate evidences a very marked shift from the predominantly reductive androgen metabolism of young animals to a predominantly oxidative androgen metabolism in aged animals. Dorsolateral prostatic complex evidences a pronounced aging-associated diminished reductive androgen metabolic potential; however, the increased production of oxidized androgen metabolite which characterizes the ventral prostate of aged animals is not detectable in the dorsolateral prostate. It is perhaps significant that the most profound aging-associated changes in androgen receptor content and metabolism occur in that lobe of the rodent prostate which recently has been demonstrated to spontaneously develop adenocarcinoma.

Evaluation of prostatic androgen metabolic potential with tissue from young and aged pure-bred beagles indicates the per gm metabolic capacity to be very much less than that of the rodent prostate and that the organ is homogeneous with regard to androgen metabolic capability. Moreover, preliminary evaluations indicate that in complete opposition to the rodent, the testosterone metabolic capability of the young canine is predominantly oxidative. In addition, aging in the dog apparently results in a very pronounced shift to reductive androgen metabolism.

Significance to Biomedical Research and the Program of the Institute: A major goal of the National Cancer Institute is determination of cause and early detection of risk of cancer. Elucidation of endocrinologic changes in prostatic homeostasis which may be causally related to the pathogenesis of

prostatic carcinoma will greatly facilitate the achievement of these gaols. Identification of causative factors will allow for meaningful endocrinologic manipulation in an attempt to reduce the mortality and morbidity of prostatic carcinoma.

Propose Course: This project will continue to define the changing endocrinology of the aging canine prostate and initiate similar studies of human prostatic tissue. These evaluations will include delineation of prostatic androgen receptor content and distribution and androgen metabolic potential.

Date Contract Initiated: June 28, 1972

Current Annual Level: $88,346

STANFORD UNIVERSITY (NO1-CP-55649)

Title: The Role of Hormonal Induction of Permanent Cellular Alterations During Perinatal Exposure on Carcinogenesis

Contractor's Project Director: Dr. Gerald R. Cunha

Project Officer (NCI): Dr. Jerry M. Rice

Objectives: To determine the role of the epithelio-stromal interaction in induction and perpetuation of ovary-independent persistent vaginal cornification and subsequent neoplasia and to determine the role of the epithelio-stromal interaction in normal pre- and postnatal morphogenesis of the vagina, uterus, cervix, ductus deferens and prostate of mice.

Major Findings: Although this contract is in an early stage, stromal induction of abnormal epithelial differentiation has been demonstrated in recombinants of vaginal stroma from estrogenized neonatal mice associated with untreated vaginal epithelium. In this recombinant the untreated epithelium is hyperplastic and in some cases exhibits parakeratosis, despite the fact that these grafts are grown in ovariectomized hosts.

Significance to Biomedical Research and the Program of the Institute: The normal morphogenesis and cytodifferentiation of many embryonic structures has been shown to be dependent on an interaction between epithelium and mesenchyme. In addition, maintenance of adult structure and function also appears dependent on such an interaction, and some investigators have suggested that carcinogenesis may occur as a result of an alteration of the interaction between epithelium and stroma. This hypothesis, for technical reasons, has not been confirmed. However, use of the animal vaginal tissue as a model system provides a technically-feasible approach to this fundamental question. Thus, stromal control of epithelial neoplasia can now be evaluated. Application of data on stromal control of normal and abnormal epithelial morphogenesis could provide the basis for developing new therapeutic approaches to the control of mammary, cervical, vaginal, prostatic, and perhaps other carcinomas.

Proposed Course: Following evaluation of current series of recombinations of epithelium and stroma from vaginas from estrogenized and untreated female mice, these experiments will be repeated and the grafts allowed to grow in ovariectomized hosts for 12 to 18 months to determine if stromal induction of epithelial hyperplasia and parakeratosis will lead to epidermoid carcinoma of the untreated vaginal epithelium as has been reported in tact vaginas of neonatally estrogenized mice. In addition, an extensive series of heterotypic recombinations between epithelium and stroma from various regions of the reproductive and integumentary systems will be constructed in order to determine the roles of epithelium and stroma in the normal pre- and post-natal development of the reproductive tract.

Date Contract Initiated: November 1, 1974

Current Annual Level: $72,589

TEMPLE UNIVERSITY (NO1-CP-33262)

Title: Biochemical and Morphologic Components of Hepatic Carcinogenesis

Contractor's Project Director: Dr. Emmanuel Farber

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: To develop a bioassay technique for rapid screening of potential hepatocarcinogens using the hyperplastic nodule as the end-point; to establish the relationship of nodular hyperplasia to the origin of hepatocellular neoplasms; to characterize biochemically and morphologically subcellular markers of the hyperplastic nodule and to determine their occurrence in nodules produced with diverse carcinogens; and to obtain tissue in larger amounts for these studies. The test system should thus provide a means for rapid identification of hepatic carcinogens and offer insight into molecular mechanisms of pathogenesis.

Known or suspected hepato-carcinogens and hepato-toxins are fed to Wistar or Fischer strain rats in a basal diet of known composition and the production of hyperplastic nodules and/or malignant hepatomas are noted. Relationship of nodular proliferation to tumorigenesis are studied by biochemical, metabolic and histological observations at the light and electron microscope levels and by the isolation by cell cultures of altered cell populations induced by carcinogens in vivo.

Major Findings: A new protein antigen (PN antigen) has been found to be a highly specific marker for hepatocytes in hyperplastic nodules in liver during hepatocarcinogenesis induced by 2-acetylaminofluorene (2-AAF) or ethionine. This new marker is present so long as the putative premalignant cell population remains fetal-like in organization. Early, on removal of the carcinogen, the population undergoes maturation to adult-like liver and this is associated with the disappearance of the PN antigen. Partial purification of the antigen has been accomplished, using sephadex G 200, DEAE-cellulose and preparative polyarcylamide gel electrophoresis. A new regimen is being

developed to demonstrate altered hepatocytes early in carcinogenesis. The basis for this approach is the previous finding that the putative premalignant cells are much more resistant to the cytotoxin action of many carcinogens.

Significance to Biomedical Research and the Program of the Institute: These new findings have importance in our further understanding of mechanisms of cancer induction by chemicals and offer a real possibility to distinguish preneoplastic liver cirrhosis in humans from the many cases of cirrhosis not related to liver cancer. In respect to the liver, it offers the most convincing evidence for the preneoplastic role of hyperplastic nodules in the induction of hepatocellular carcinoma. Thus, these new observations may well be significant in the following aspects of the objectives of the National Cancer Institute; (a) determination of cause; (b) early detection of risk; (c) early diagnosis of lesions and (d) the effective prevention of human cancer.

Proposed Course: (a) To pursue in increasing depth the nature and significance of the new specific marker for preneoplastic liver tissue in mechanisms of carcinogenesis and in the analysis of human liver cancer and liver cirrhosis; (b) to begin to explore the possible presence of analogous markers in preneoplastic cells in other organs or tissues such as skin, pancreas and colon; (c) to develop a better scientific basis for the rapid bioassay of carcinogenesis using end-points other than the initial interaction with target macromolecules; and (d) to develop a method to identify and isolate altered hepatocyte populations early in carcinogenesis as a basis for a possible new rapid bioassay for carcinogens.

Date Contract Initiated: January 20, 1964

Current Annual Level: $48,238

TENNESSEE, UNIVERSITY OF (NO1-CP-33369)

Title: Maintenance of Organ Explants from Rodent Pancreas

Contractor's Project Director: Dr. Leonard Murrell

Project Officer (NCI): Dr. Stuart Yuspa

Objectives: The technique of organ culture is increasingly being used to bridge the gap between cell culture and in vivo conditions. The ability to maintain a differentiated tissue in culture permits direct hisotological and/or biochemical assessment of the effect(s) of chemical carcinogens on the tissue itself, and, simultaneously, evaluation of products released by cultured explants. It is becoming apparent that each organ has specific culture requirements. In addition to the requirements common to all in vitro environments (temperature, humidity, etc.) it is now recognized that many cultured organs require rigorous control (at specific levels) of pH, osmolality, hormonal milieu, and other system dependend factors, such as oxygen concentration. Therefore, any realistic approach to maintaining

pancreas in culture must include detailed assessment of each of these conditions, as well as evaluation of their possible interactions. The present project attempts to elucidate some of the factors necessary to maintain rodent pancreas--in organ culture. After development of appropriate culture conditions, the organ cultures will be used in studies of chemical carcinogenesis of the pancreas.

Major Findings: Classic organ culture methods are not suitable for mature mammalian pancreatic acinar tissue. Therefore, a circumfusion culture system capabable of exchanging culture medium, in whole or in part, at predetermined intervals, was designed. Survival of cultures is monitored by evaluation of (a) amylase released into the culture medium, (b) amylace content of cultured explants, and (c) by morphology (at the light microscopic level) of cultured explants. Currently, this work employs a serum free chemically defined culture medium, and a circumfusion cycle which replaces 50% of the culture medium at 3 hour intervals. Other culture conditions (gas phase oxygen tensions, medium pH, explant size, etc.) are maintained at levels established as optimal in earlier phases of the project. Addition of hydrocortisone or corticosterone to culture medium results in markedly enhanced survival for culture periods up to 8 days. Both histological structure and tissue amylase contents are similar to those of appropriate uncultured control tissues when glucocorticoids are included in the medium. Further, amylase contents of tissues respond to variation in glucocorticoid concentration in a dose dependent manner. The majority of the experimental work has employed pancreatic explants obtained from rats ranging in age from birth to 21 days postnatal. Initial experiments suggest that similar methods are suitable for pancreas obtained from neonatal guinea pigs.

Significance to Biomedical Research and the Program of the Institute: Cancer of the pancreas, sixth in rank among fatal human cancers, causes over 19,000 deaths per year in the United States. Since the Institute began its pancreatic carcinogenesis programs, much has been learned about the disease, and some animal models have been developed. However, there are no fully developed in vitro approaches to the study of potential carcinogenic transformation of pancreatic acinar cells. This project is one of several initiated to develop animal models and experimental approaches to studies of the etiology and pathogenesis of this major form of human cancer.

Proposed Course: Continuing experiments involving manipulation of medium circumfusion conditions, and variations in medium composition, are directed toward increasing the period of tissue viability with retention of in vivo-like structure during culture. Mitotic potential of cultured tissue will be evaluated by autoradiography following addition of tritiated thymidine to culture media; functional integrity of secretory mechanisms of cultured tissue will be examined by challenge with appropriate secretogogues. Feasibility studies designed to evaluate the transplantation potential of cultured pancreatic acinar tissue (a possible means for analyzing long term effects of in vitro carcinogen treatment) are contemplated. Finally, efforts to determine the suitability of circumfusion culture methods for pancreatic acinar tissue of other rodent species will be conducted in order to expand the applicability of this experimental model.

Date Contract Initiated: June 29, 1972

Current Annual Level: $76,094

WEST VIRGINIA UNIVERSITY (NO1-CP-43230)

Title: Relationships of Pituitary Hormones and Androgen on Prostate Metabolism

Contractor's Project Director: Dr. John A. Thomas

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: The major objective of these investigations is to determine how pituitary hormones, such as prolactin, affect the growth and biochemical regulation of different lobes of the prostate glands. This study involves a comparison of several different species with regard to prostate physiology. Since the prostate gland is androgen dependent, these studies will be concerned with the interaction of these steroids and/or non-gonadotropic hormones. Particular emphasis will be placed upon the effect(s) that prolactin exerts upon testosterone metabolism. Furthermore, this project shall examine the effects of carcinogens (e.g. ethylnitrosourea (ENU), dimethylbenzanthracene (DMBA), and methylcholanthrene (MC) on rat prostate cell populations. Prostate explants are to be treated with direct and indirect acting carcinogens in culture and then to implant these carcinogen-treated prostates from the in vitro environment to syngeneic hosts. The results obtained from these studies may provide further insight into the biochemical processes involved in normal and abnormal growth of the prostate gland.

Major Findings: The role of age of prostate in the organ's response to hormones is beginning to evolve. Young rats (280 gms) when treated with low doses of prolactins failed to exhibit any significant increases in RNA, DNA or protein levels, whereas older rats responded with evaluations in these parameters. It thus appears that the prostate gland of the older animal is more sensitive to exogenous prolactin. The dorsal lobe appears to be the primary responsive portion of the prostate. Zinc increases the conversion of tritiated testosterone to dihydrotestosterone and to androstandiol. The in luence of zinc on the interation of prolactin with androgens is a current goal.

Significance to Biomedical Research and the Program of the Institute: Despite the fact that there is a very high incidence of adenocarcinoma of the prostate, no ideal animal model has yet been disclosed in which to study this form of cancer. The disclosure and full elucidation of such an animal model would be a significant contribution to understanding of human adenocarcinoma of the prostate. Not only would a knowledge of the pathological biochemistry be of value, but efforts could then be focused upon searching for a useful therapeutic method of controlling this disease which afflicts a substantial number of elderly males.

Proposed Course: A proposed series of experiments will investigate the

incorporation of thymidine-H^3 into prostatic DNA and uridine-H^3 into prostatic RNA. Such experiments will not only involve different doses of exogenous prolactin (and/or testosterone or dehydrotestosterone), but will study the synthesis of RNA and DNA in vitro.

A series of experiments involving prostatic explants have been underway for about one month. Prostate glands (various lobes - anterior, dorsal or ventral) from twelve-week-old donor animals have been transplanted into 24-day-old recipient animals. The recipients or host groups pf animals were (a) males implanted with testosterone pellets, (b) males receiving no exogenous testosterone, (c) females implanted with testosterone, and (d) females receiving no testosterone. Although some mortality in this series of experiments has occurred at this early date, the males implanted with testosterone pellets have begun to exhibit palpable prostatic transplants.

Another series of proposed experiments involve the injection of carcinogen into young mature male rats. Carcinogens were recently injected into groups of animals, and they will be sacrificed 6, 12, and 24 weeks post-injection for examination of sex accessory gland morphology and biochemistry (steroidogenesis and nucleic acid metabolism). ENU or DMBA will be investigated for their ability to induce enhanced prostatic growth as evidenced by increased RNA and DNA metabolism.

Date Contract Initiated: June 28, 1972

Current Annual Level: $111,066

WRIGHT STATE UNIVERSITY (NO1-CP-43231)

Title: Cell Culture Development of Human and Guinea Pig Pancreas Cells

Contractor's Project Director: Dr. Robert J. Hay

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: To establish reproducible techniques for cultivation of pancreatic epithelial cells. Such cultures can then be used for studies on chemical carcinogenesis.

Major Findings: Techniques for obtaining colonial aggregate cultures of pancreatic acinar cells have been described. Extensive trial studies have been continued to identify agents or conditions which may stimulate such cultured cells to resume active protein synthesis and secretion. Most of the common hormones and secretogogues have been shown to be ineffective in this regard at least under the conditions imposed. Experiments involving reimplantation of colonial aggregates back into host animals, however, appear to have been successful. Aggregate colonies after 72 hours in culture were placed in standard diffusion changers. These were then inserted into peritoneal cavities of host guinea pigs. After an additional 48 hours (120 total from dissocation) the chambers were removed and colonial aggregates on the membranous walls were stained by the Mallory Heidenhain method. Cells

containing zymogen droplets were observed. These results suggest that cultured acinar cells can be activated if appropriate stimuli are applied.

The synthesis of DNA by acinar cells in colonial aggregate cultures has also been investigated both by standard isotope incorporation and by autoradiography. Thymidine uptake per unit cell decreases dramatically during the first 10 days. This is a reflection of a decline in the percentage of cells capable of such incorporation as a function of time. These findings are reminiscent of those obtained with human and other diploid cells in vitro, and are presumed to reflect as yet unresolved inadequacy in culture conditions.

A strain of epithelial cells has been isolated from the pancreas of a rat treated with azaserine. The cells comprising this strain have undergone over 25 population doublings at this writing and show no sign of diminished proliferative rate. Studies of cell ultrastructure reveal small but distinct zymogen-like droplets in the cytoplasm.

Significance to Biomedical Research and the Program of the Institute: The development of model systems for the cultivation of pancreatic exocrine and duct cells would provide powerful tools for the study of carcinogenesis. Definition of the factors that promote continued proliferation and function in such systems would also contribute to basic understanding of pancreatic epithelial cell physiology.

Proposed Course: Additional study is in progress involving implantation of diffusion chambers with pancreatic colonial aggregates into host animals. Absolute proof of reactivation requires that initial cells be labelled and that cells on diffusion chamber walls produce high levels of pancreatic-specific enzymes. Further definition of this phenomenon may also be useful in identifying the conditions which promote activation.

The pancreatic epithelial cell strain from the azaserine treated rat will be characterized in terms of karyology, proliferative potential and amylase content.

Attempts are being initiated to isolate pancreatic duct cells by differential tissue dissociation and/or by isokinetic sedimentation through ficoll gradients.

Date Contract Initiated: June 28, 1972

Current Annual Level: $56,679

SUMMARY REPORT

BIOLOGY AND IMMUNOLOGY SEGMENT

July 1, 1974 through June 30, 1975

The major goals of the Biology and Immunology Segment are (1) to devise fast and reliable assays for the detection of chemical carcinogens, (2) to develop immunologic techniques that may be useful in studies on chemical carcinogenesis and (3) to learn how to use immunologic maneuvers to inhibit carcinogenesis. During this year five contracts, which were developed and monitored by the Biology and Immunology Segment, were transferred with funds to the Tumor Immunology Program of the Division of Cancer Biology and Diagnosis. They are (1) Trudeau Institute, Inc, (NO1-CP-23221); (2) Mallory Institute of Pathology Foundation, (NO1-CP-33264); (3) University of Illinois, (NO1-CP-23205); (4) Scripps Clinic and Research Foundation, (NIH-NCI-E-43277), and (5) A portion of ERDA-NCI Interagency Agreement (Oak Ridge National Laboratory) (YO1-CP-50200).

In Vitro Carcinogenesis

At Biolabs, Inc. (NO1-CP-45615), the primary objective is to study in vitro assay systems which would be used for identification of potential carcinogens and to use these systems to evaluate the role of chemical carcinogens in promoting (enhancing) viral carcinogenesis.

Diverse chemical carcinogens enhance the frequency of transformation by simian adenovirus, SA7. The effect of adding various chemical carcinogens at times after virus inoculation and cell transfer was determined. Cell survival was quantitated by cloning procedures and SA7 transformation counting foci growing under 0.5% agar overlay. The carcinogens methylmethanesulfonate (MMS), 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) and N-acetoxy-2-fluorenyl-acetamide (N-acetoxy AAF) caused maximal enhancement of SA7 transformation when added 2 hours before, or 5 hours after, virus addition. A slight increase was also observed when these chemicals were added 24 hours after virus, but not at 48 hours. Alternatively, the polycyclic hydrocarbons benzo[a]pyrene (BP), 3-methylcholanthrene (3-MCA), and 7,12-dimethylbenz[a]anthracene (DMBA) increased the viral transformation frequency only when added 4 to 24 hours prior to virus, but inhibited transformation when added at 4, 8, 12, or 24 hours after virus. In contrast to both of the above observations, enhancement by treatment of cells with caffeine or 6-acetoxy-benzo[a]pyrene was maximal when chemical was added 5 hours after virus, but was minimal or absent when added prior to virus. Thus, maximal chemical enhancement of adenovirus transformation depends upon the sequence of addition of virus and chemical. Chemicals that enhanced viral transformation may or may not induce DNA breaks or repair synthesis.

Clones of transformed hamster embryo cells were characterized by (1) content of virus-specified antigens; (2) cloning capacity in soft agar; and (3) tumorigenicity in hamsters. All clones contained SA7 "T" antigen (detectable by complement fixation). There was no correlation between antigen levels

and type of chemical pretreatment. Tumor transplant rejection data indicate that transformed clones derived from combined treatment contain transplantation antigens similar to those found in clones transformed by SA7 alone. All clones transformed by SA7, or by the interaction of chemical and virus, formed colonies in soft agar. The quantitative differences in cloning efficiency were not related to treatment. All clones were tumorigenic, but some selected from SA7 transformed cells that had been pretreated with 3-methylcholanthrene, 7,12-dimethylbenz[a]anthracene, or benzo[a]pyrene exhibited increased tumor incidence and shorter latent period compared to cells transformed by SA7 alone. The degree of tumorigenicity could not be predicted by relative cloning efficiency in soft agar.

At Ohio State University Research Foundation (NO1-CP-43276), the objective is to evaluate human cell culture systems as in vitro assays for chemical carcinogenesis. This includes identification of potential carcinogens, evaluation of the role of hormones as inhibitors or promoters of in vitro carcinogenesis and investigation of the role of nononcogenic and oncogenic viruses in promoting chemical carcinogenesis in human cells. Alterations of growth characteristics including morphologic changes of human cells in culture have been obtained with several carcinogens. Such cultures possessed increased cell density, greater doubling potential, losses of contact inhibition, increased cloning efficiency and ability to grow under or in soft agar. These effects were enhanced by the addition of 17-β-estradiol or cortisone acetate. Some cell populations subjected to multiple treatment by chemical carcinogens have extended life-spans but have not produced tumors.

Transformation of Detroit-550 cells by SV_{40} was enhanced by pretreatment with BP, MNNG, 4NQO and N-acetoxy-AAF but not by MCA or DMBA. In contrast to these results, promotion was not achieved by DMBA (0.5% μg/ml) or with MCA-(5.0 μg/ml). MCA significantly inhibited transformation when added 2, 6 or 24 hours after virus. DMBA also diminished transformation rates to a lesser degree when added after virus. The ability of these chemicals to induce damage to cellular DNA, alter its repair or influence scheduled DNA synthesis differs in Detroit-550 cells. These differences are reflected in their effects on transformation of human cells by SV_{40}. Substitution of an RNA-virus, Feline Sarcoma virus for a DNA virus also results in an increased transformation frequency when the cells are pretreated with a chemical carcinogen.

Immunology

A contract is devoted to studies on the detection, significance and usefulness as tumor markers of alpha-fetoglobulins of rats and guinea pigs. Alpha-fetoglobulin increases in the circulation of patients and animals suffering from primary hepatomas. Studies on rat alpha-fetoglobins showed that increased production of alpha-fetoglobulin is not a necessary consequency of malignant transformation of liver cells; on the other hand, alpha-fetoglobulin appears in the serum of rats within one week after feeding an oral hepatocarcinogen. The increase is obtained with a dose of carcinogen that produced no morphological changes in the liver and that is insufficient to induce hyperplastic or neoplastic changes. Alpha-fetoglobulin is apparently produced for only a limited time during the cell cycle of hepatoma cells (University of California

[at San Diego] [NO1-CP-33403]). The function of macrophages was studied in two contracts. In one the in vivo and the in vitro effects of chemical carcinogens on the synthesis of complement components were studied. Nitrosamines that inhibit in vitro C4 and C2 synthesis within 24 hours after in vivo administration do not inhibit the in vitro production of these components if the carcinogen is administered in vitro. Chemotaxis of macrophages was also inhibited by in vivo injection of nitrosamines. Inhibition of C2 and C4 synthesis by dimethylnitrosamine in vivo was negated when given together with non-carcinogenic nitrosamines or in the presence of a sterile abscess induced by turpentine (Children's Hospital Medical Center [NO1-CP-45612]). In another study, it was found that chemotaxis of human macrophages is greatly reduced in about one-half of patients suffering from cancer. Improved chemotaxis was noted in patients with recurrent malignant melanoma after treatment with BCG (Duke University Medical Center [NO1-CP-33313]). Studies based on the fact that some carcinogens are allergens and haptens are being done at contract sites. Progress has been made in finding suitable coupling agents for carcinogens so that they become immunologically active in vitro (University of Texas at San Antonio [NO1-CP-43353]) and in vivo (Brandeis University [NO1-CP-23243]). In vitro tests are now being developed that may permit the detection of cellular immune reactivity to carcinogens as a measure of previous exposure to these agents. Antibodies have been produced to several carcinogens, insecticides and anti-neoplastic agents that may make it possible to develop tests of humoral immunity to detect prior exposure to the carcinogen. The principle of this kind of detection system has been established with non-carcinogens at Brandeis University by showing that nicotine and cotinine can be detected in the serum of smokers by use of specific antibodies to these chemicals. At (Case Western Reserve University [NO1-CP-43354]) animals are rendered immunologically tolerant to a chemical carcinogen and then tested for susceptibility to tumorigenesis by that carcinogen. Tolerance is induced by oral feeding of carcinogen. Oral feeding of carcinogens so far has produced no tumors in guinea pigs, and tolerance to carcinogens has had no detectable effect on tumorigenesis. Intravenous infusions of DMBA in fat emulsion has produced mammary tumors in guinea pigs. Immunologic methods are being tested for their capacity to inhibit mammary tumorigenesis. At (Temple University [NO1-CP-33200]) guinea pigs whose skin has been painted with DMBA for more than one year have developed squamous cell carcinoma resembling the human disease. They have also developed melanotic lesions. These carcinomas are focal lesions that do not obliterate the melanotic lesions. Several animals have developed invasive melanoma with lymph node metastases. The next phase of this project will be to determine whether any of the stages of carcinogenesis can be inhibited by immunologic methods.

Studies are underway at the (University of Utah [NO1-CP-45600]) to determine the effects of chronic treatment with cyclophosphamide, methotrexate, azathioprine, prednisolone, azathioprinze plus prednisolone, and antilymphocytic serum on benzopyrene and ultraviolet (UV) skin carcinogenesis in mice and to investigate the immunological aspects of UV carcinogenesis in mice. Appropriate doses of benzo[a]pyrene and UV light to use in studies combining treatment with these carcinogens and immunosuppressive drugs have been defined. Dose and regimen for each of the immunosuppressive agents which permit chronic drug administration without excessive toxicity have been determined. The

effects of the immunosuppressive drugs on skin allograft rejection have been investigated. It has been found that tumors induced by UV light exhibit an unusually high degree of antigenicity,and that they are not detectably cross reactive when tested by transplantation techniques. The findings on the antigenicity of UV-induced tumors provide a unique opportunity to investigate the mechanism which enables highly antigenic tumors to escape immunologic destruction in the primary host.

Two new contracts have been initiated to study the effects of chronic administration of a low dose of carcinogen to experimental animals on several aspects of their immune system (Ohio State University [NO1-CP-53329] and Public Health Research Institute of the City of New York, Inc. [NO1-CP-55679]).

CONTRACT NARRATIVES

BIOLOGY AND IMMUNOLOGY SEGMENT

July 1, 1974 through June 30, 1975

BIOLABS, INC. (NO1-CP-45615)

Title: In Vitro Study of the Nature of the Interaction Between Chemical and Viral Carcinogens

Contractor's Project Director: Dr. Bruce C. Casto

Project Officer (NCI): Dr. Joseph A. DiPaolo

Objectives: The specific objective of this contract is to study the process whereby chemical carcinogens promote viral oncogenesis and to determine if oncogenic and non-oncogenic viruses may similarly promote oncogenesis by known chemical carcinogens.

Major Findings: This study has established that treatment of cells with chemical carcinogens will increase the frequency of viral transformation in vitro. Over 60 chemicals, including polycyclic hydrocarbons and alkylating agents, have been tested for their capacity to enhance viral (Simian adenovirus, SA7) transformation of hamster cells, induce single-strand breaks in cell DNA, and stimulate unscheduled DNA (repair) synthesis.

Carcinogenic polycyclic hydrocarbons enhanced adenovirus transformation, but the non-carcinogens did not. Enhancement has been induced by MNNG, Ac-AAF, MMS, EMS, MAM, MOCA, and AFB_1, but not by AAF, DEN or DMN. Other compounds such as cytosine arabinoside, caffeine, hydroxylamine, cyclohexylamine, β-propiolactone, propane sultone, β-napthylamine and dichlorobenzidine, also enhance adenovirus transformation of hamster cells.

The sequence of addition of virus and chemical was found to be critical to demonstrate enhancement by many of the compounds. The polycyclic hydrocarbons BP, 3-MC, and DMBA, in addition to AFB_1, inhibited transformation when added after SA7 and cell transfer. The chemicals MMS, EMS, MAM, MNNG, Ac-AAF, and β-PL enhanced transformation when added before or after virus, whereas ara-C, caffeine, and 6-acetoxy-BP demonstrated greater enhancement when added after virus adsorption and cell transfer.

Chemicals which induce enhancement by pretreatment of cells, were found to be more effective if virus was added immediately after treatment. Maximal enhancement was observed with MMS, Ac-AAF, MNNG and DMBA when virus was added as above; enhancement induced by MMS persisted less than 24 hours, by MNNG and Ac-AAF for 48 to 72 hours and by DMBA for 72 hours or more.

All compounds which were shown to induce single-strand DNA breaks, and/or stimulate cell DNA repair synthesis, enhanced transformation by SA7. However, enhancement was not restricted to chemicals which cause either of the above, since BP, DB[a,c]A, and caffeine stimulate SA7 transformation, but do not

produce demonstrable DNA breaks or repair synthesis.

DNA from cells treated with MMS, contained up to 4 times more viral DNA than DNA from SA7 inoculated control cells. Clones of SA7 transformed cells derived from carcinogen treated or non-treated hamster embryo cells contained an equivalent amount of viral DNA in cell DNA. Treatment of cells with benz[a]anthracene (BA) or 7,8-benzoflavone (ANF) inhibited cell death and enhancement by BP or 7,12-DMBA when added simultaneously to hamster embryo cells. Both ANF and BA reduced the toxicity of 3-MC, but had less effect on the enhancement of virus transformation by 3-MC. The protective effect of BA and ANF against BP and 7,12-DMBA was essentially gone by 24 hours after treatment, as well as the inhibition of enhancement. At several concentrations of BP and 7,12-DMBA where the toxicity of the chemical was decreased by BA or ANF, the actual number of SA7 foci increased several fold.

Vitamin A had no effect on the enhancement of SA7 transformation by BP. Similarly, neither caffeine nor chloroquine had any inhibitory or augmentative effect on enhancement induced by Ac-AAF or MMS.

Cells transformed by the interaction of chemical carcinogens and SA7, and cells transformed by SA7 alone, contained SA7 "T" antigen in equivalent amounts, but none of the cell cultures was found to contain hamster C type virus antigen. SA7 transformed cells selected from HEC treated with chemical and virus contained transplantation antigens similar to those found in cells transformed by SA7 alone.

All SA7 transformed cells, whether derived from carcinogen treated HEC or from control cells, cloned in soft agar and were tumorigenic in weanling hamsters. Certain cell lines selected from SA7 transformed foci in HEC that were pretreated with MC or DMBA appeared to have an increased tumorigenicity over parallel cultures transformed by SA7 alone.

Hamster cells transformed by 3-MC and subsequently by SA7 cloned more efficiently in soft agar and were more tumorigenic than cells transformed by SA7 alone. Antiserum to the doubly-transformed cell lines was cytotoxic for the homologous cells, the parent chemically transformed line, and for several SA7 transformed lines. The morphology of the double transformants was intermediate to that of 3-MC and SA7 transformed cells.

Transformation of hamster cells to form discrete foci was accomplished with 3-MC, BP, DB[a,h]A, AFB_1, EMS, MMS, and MNNG. Transformation by 3-MC was more efficient if cells were treated for 6 days as opposed to treatment for 1 or 3 days, and more transformed foci could be demonstrated when 50,000 cells were treated with 3-MC in contrast to that obtained when fewer or larger numbers of cells were plated. Low passage chemically-transformed cells were unlike high passage cells with respect to cloning in soft agar, tumorigenicity in hamsters, sensitivity to polyanions and growth in 1% serum.

Significance to Biomedical Research and the Program of the Institute: In view of the various hypotheses concerning chemicals, viruses, and cancer, and due to the inherent difficulties in studies in the intact animal, it is advantageous to study the various interactions in a controlled in vitro

system. This would establish not only the influence of oncogenic and non-oncogenic viruses in promoting chemical carcinogenesis, but also the role of chemicals in promoting viral carcinogenesis. Sensitive quantitative assays, such as those developed under this contract, may be used to detect potential carcinogenic or mutagenic chemicals in the environment.

Proposed Course: Diverse chemicals that are important to the human environment, including pesticides, herbicides, and industrial chemicals, will be considered for their ability to influence virus transformation. The nature of the persistence and inhibition of enhancement by chemical carcinogens will be the subject of further studies. An attempt will be made to correlate the persistence of enhancement with DNA breaks, and where such breaks are not found an attempt will be made to determine if the chemical carcinogen remains bound. The factors influencing the quantitative transformation by polycyclic hydrocarbons will be determined by using compounds known to influence the toxicity of these carcinogens as well as by altering the type of cells at risk, the concentration of the chemical compound, and also the length of time the cells are exposed. An evaluation will also be made to determine if the phenomenon of enhancement observed will occur with other types of host cells and viruses. The influence of different types of viruses on the frequency of transformation by chemicals will be explored using a quantitative focus assay that does not require the use of feeder cells. Cells transformed by the combined action of chemical and virus will be characterized and compared to cells transformed by virus or chemical alone. Preliminary evidence, from soft agar cloning experiments and tumorigenicity in animals, indicate that the chemical may contribute additional properties to the cells as well as stimulate viral transformation. Thus, the clones that have been transformed will be studied for antigenic changes and chromosome alteration with the hope that these parameters may be correlated with an altered tumorigenicity.

Date Contract Initiated: June 9, 1971

Current Annual Level: $232,104

BRANDEIS UNIVERSITY (NO1-CP-23243)

Title: Production and Detection of Antibodies to Chemical Carcinogens and Other Small Molecules

Contractor's Project Director: Dr. Helen Van Vunakis

Project Officer (NCI): Dr. Herbert Rapp

Objectives: To obtain antibodies that react specifically with chemical carcinogens and other small molecules of interest in cancer research. The antibodies will be used as reagents to develop immunoassays capable of detecting the corresponding haptens in body fluids and tissues. Haptens attached to appropriate carriers will be used as antigens to detect presence of antibodies in the serum of animals and humans. These reagents will be tested for their suitability for the detection of exposure to environmental

agents that may be related to cancer causation.

Major Findings: Radioimmunoassays (RIAs) have been developed for the detection of picomoles of nicotine and two of its metabolites (cotinine and γ-(3-pyridyl)-γ-oxo-N-methylbutyramide); the polycyclic aromatic hydrocarbons (benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene); the mycotoxin (aflatoxin B1); the polycyclodiene-polychlorinated insecticides (dieldrin and aldrin) and the antineoplastic agents (adriamycin and daunomycin). These assays are sensitive and specific and can be used to determine levels of the parent compound or metabolite in physiological fluids, tissue extracts or other material, for pharmacokinetic studies, to follow metabolic conversions, and to partially characterize enzyme systems. Often samples can be assayed directly without prior extraction or isolation of the compound.

(a) Nicotine and metabolites: Although the metabolism of nicotine is complex, the specificities of the antibodies allow quantitative determination of nicotine, cotinine and γ-(3-pyridyl)-γ-oxo-N-methylbutyramide in the presence of each other and several other metabolites. In smokers' sera, cotinine, the major and a relatively long-lived metabolite, is found in concentrations ranging up to 600 ng/ml. The nicotine content usually does not exceed 50 ng/ml and its half-life is short. Probably due to differences in smoking habits and/or other physiological factors, the correlation between cotinine levels in blood and the nicotine contained in the cigarettes an individual claims to smoke daily is poor. It may be advantageous to assay for cotinine in epidemiological studies designed to relate the effects of smoking to health. These RIAs have also been used to monitor the oxidation of nicotine to cotinine and cotanine to the oxamide by enzymes present in rabiit liver extracts. The results were confirmed by high-pressure liquid chromatography.

The absorption of nicotine has been implicated in the etiology of "green tobacco sickness" which occurs among young men who have considerable contact with the uncured tobacco leaf during the harvesting of tobacco and who are non-smokers. Analysis of their urine indicated that cotinine was present in amounts greater than that found in the urine of novice smokers who smoked three cigarettes containing 1.2 mg nicotine/cigarette. Since this metabolite is not found in tobacco leaf, it cannot be a source of contamination.

Although other compounds that may be present in tobacco are not ruled out, the fact that the symptoms resemble that of nicotine intoxication and the finding of substantial amounts of the metabolite in urine, argues against this.

The nicotine and cotinine analogs of DPN and TPN have been isolated and identified from incubation mixtures containing nicotine or cotinine, DPN or TPN, and a source of DPNase. They cannot replace the coenzymes in DPN or TPN-specific dehydrogenases. Competitive inhibition with respect to DPN or TPN has been observed in some cases.

(b) Polycyclic aromatic hydrocarbons: Two differently substituted derivatives of BP and a 7,12-DMBA derivative were used to prepare conjugates for immunization. While the antibodies react more effectively with the homologous haptens, they cross react to varying degrees with structurally related compounds. Analyses of sera indicate that BP and DMBA, if they occur at all, are

present in concentrations of less than 500 pg/ml. If immunoassays which depend upon the host's cellular responses are required to monitor populations for exposure to PCHs, the serological activities described may serve as a guide for evaluating the specificities of such responses. Also multivalent conjugates, such as those prepared for this study, may be necessary to elicit such responses in sensitized animals.

(c) Aflatoxins: Suitably functionalized derivatives of Afla B1 and an analog of Afla B1 (5,7-dimethoxycyclopentenon(2,3-c)coumarin) were used to prepare conjugates for immunization. The specificities of the antibodies decreased in the order Afla B1 B2 G1 G2 and as little as 60 pg of Afla B1 can be measured. Sterigmatocystin, warfarin and coumarin are poor inhibitors. Use of the analog has practical advantages since it minimizes contact with the highly toxic and carcinogenic Afla B1 during syntheses. These assays are sufficiently sensitive and specific for use in determining levels of the mycotoxins in contaminated foodstuffs and biological samples.

(d) Dieldrin and aldrin: A radioimmunoassay for dieldrin and aldrin has been developed which can detect picomole levels of either compound. The specificity of the antibodies was determined with respect to several other organochlorine insecticides including DDT, 2,4,5-T and decachlorobiphenyl (a PCB standard). These and other pesticides which may be found in samples containing dieldrin and aldrin should not interfere with the assay.

(e) Adriamycin: An RIA for adriamycin, a drug which shows considerable promise in cancer chemotherapy, has been developed. Pharmacokinetic studies on rabbits, monkeys, and 20 patients with different types of cancer have been carried out. The assay is sensitive and permits levels of the drug to be determined in plasma and urine samples taken one week after administration of the drug to patients. This assay is also applicable in estimation of levels of daunomycin, a drug closely related to adriamycin but used in patients with different types of malignancies. In addition, the specificity of the antisera permits estimation of adriamycin equivalents in the presence of bile salts which constitute an endogenous source of interference in the fluorescence assays in current use and which are especially troublesome in patients with liver dysfunction. Adriamycin and its metabolites (adriamycinone and adriamycinol) can be separated by high pressure liquid chromatography and estimated by RIA. The ratio of metabolites to parent drug differs among individual patients and in urine samples taken from the same individual at different days.

Significance to Biomedical Research and the Program of the Institute: This work may provide a way to determine the degree to which individuals in different environments have been exposed to known chemical carcinogens. The immunologic approach does not require that the carcinogen be present in the individual at the time of testing. A metabolite, which is longer lived in physiological fluids than the parent compound and/or which may be responsible for the biological effect, may provide a more reliable base for epidemiological studies. Demonstration that different groups are at risk may provide the impetus to remove certain agents from the environment. In the case of adriamycin, it may be possible to relate its toxicity to plasma levels and design regimens for individual patients which would permit the drug to be

used for chemotherapeutic purposes under conditions which minimize its toxic side effects. Assays which facilitate studies on the mechanism of action of noxious compounds will help to define the circumstances under which they might also be carcinogenic.

Proposed Course: To proceed with the objectives as stated. Rabbits or other suitable animals will be immunized with the chemical substance of interest attached to a carrier that promotes the antigenic activity of the agent. Among the agents to be used are molecules which may be carcinogens, procarcinogens or be of interest in other aspects of cancer research.

Date Contract Initiated: April 20, 1972

Current Annual Level: $113,506

CALIFORNIA, UNIVERSITY OF (at San Diego) (NO1-CP-33403)

Title: Significance and Relationship of Fetoglobulins to the Induction of Hepatomas by Chemical Carcinogens

Contractor's Project Director: Dr. Stewart Sell

Project Officer (NCI): Dr. Tibor Borsos

Objectives: The objective of this contract is to develop a sensitive new method to study the relationship between the induction of primary hepatomas and the appearance and significance of fetoglobulins.

Major Findings: Rat alpha$_1$-fetoprotein (α_1F) has been isolated and a competitive binding radioimmunoassay sensitive to 1 ng developed. Normal adult rats have a serum α_1F concentration of 0.03 µg/ml. Secretion of α_1F occurs following mitosis of neonatal liver cells, of adult liver cells following partial hepatectomy or chemically induced hepatitis, and of fetal liver cells stimulated to divide in vitro. During gestation, α_1F is produced by dividing fetal liver cells. There is rapid equilibration from fetus to mother with a fetal serum concentration of 5,000 µg/ml and maternal concentrations of 200 µg/ml at term.

Production and release of α_1F by fetal hepatocytes in vitro is coupled to cell division while that of albumin is not. α_1F is synthesized before S and released prior to M during the cell cycle.

Transplantable hepatoma cells also produce α_1F. The serum α_1F concentration may be as high as 15,000 µg/ml. However, the sera of some animals bearing transplantable hepatomas do not have elevated serum α_1F concentrations. The capacity of a given hepatoma to produce α_1F is related to growth rate, degree of differentiation and chromosomal composition.

As an α_1F producing hepatoma grows, the serum α_1F concentration increases. Removal of the tumor by surgery results in a rapid fall in serum α_1F concentration with a half-life of 24 hours. The presence of occult metastases may be

determined by rising α_1F concentrations in rats whose primary implanted tumor has been amputated. This provides a model for therapy of minimal residual metastatic tumor. Hepatomas growing in immunosuppressed hosts enlarge more rapidly, contain more cells in mitosis and produce more α_1F. Not only does the serum α_1F concentration rise at a more rapid rate, but also there is more α_1F per gram of tumor. This indicates that α_1F is synthesized by dividing tumor cells, and not presumably by quiescent tumor cells.

The production of α_1F may be the first event now detectable as an indication of the reaction of hepatocytes to the effects of a chemical carcinogen. Male Sprague-Dawley rats fed carcinogenic doses of N-2-fluorenylacetamide (2-FAA) exhibit a significant elevation of circulating α_1F within one week of feeding. This elevated serum concentration of α_1F continues for 10 weeks after the carcinogen is removed from the diet at three weeks. A gradual return to normal occurs approximately 17 weeks after cessation of the diet. Similar elevations occur in female Fischer rats which have a deficit in hydroxylation of 2-FAA and female Sprague-Dawley rats which have a deficit in esterification of 2-FAA. Higher elevations occur earlier in male Sprague-Dawley rats which have no deficit in metabolism of 2-FAA, but within 2 weeks after a 3-week dietary exposure of 0.015 to 0.06% 2-FAA, all these groups have essentially identical serum α_1F concentrations. Extremely small quantities of 2-FAA, approximately 1% of a carcinogenic dose, cause a rapid and significant rise in the serum concentration of α_1F. The effect on α_1F production of carcinogens that produce cell injury must be interpreted with caution due to production of α_1F by regenerating liver cells. Events such as partial hepatectomy or administration of galactosamine produce only a temporary elevation of serum α_1F with a return to normal within two weeks. The α_1F elevation in 2-FAA-treated rats precedes any detectable tissue alteration and is apparently unrelated to cell injury or cell division. Exposure to the hepatocarcinogen 3'-methyl-4-dimethylaminoazobenzene (3'-DAB) also produces a sustained elevation of serum α_1F. However, its non-carcinogenic analogues 2'-methyl-4-dimethylaminoazobenzene (2'-DAB) and p-aminoazobenzene (PAB) do not. Further, under circumstances wherein 2'-DAB is reported to become carcinogenic, i.e., when administered at the time of 70% hepatectomy, neither elevation of α_1F nor histologic alteration of the liver is noted.

It may be that the increase in circulating α_1F found after minimal carcinogen exposure is the result of a highly selective derepression of protein synthesis that occurs following the formation of a complex between the metabolites of the carcinogen and specific chromatin loci.

Although every animal so far tested produced α_1F after contact with even minimal doses of hepatocarcinogens, many primary carcinogen-induced hepatomas do not produce an elevation of serum α_1F. Either there is a subsequent change in the preneoplastic α_1F-producing cell that occurs prior to irreversible neoplastic alteration or it is possible that the hepatocytes originally stimulated by the carcinogen to produce α_1F are not necessarily the same cells that are the progenitors of the hepatoma produced by more prolonged carcinogen exposure.

Significance to Biomedical Research and the Program of the Institute: The

primary goal of the Carcinogenesis Program is to prevent cancer in man by identifying carcinogenic compounds and formulations that may affect humans, as well as by identifying the mechanism of action of known carcinogens. Both these approaches require extensive bioassay and other analytical technologies, as well as a continuing vigorous program for refinement and innovation of the same technologies. This contract applies immunochemical techniques to the detection and to the study of the significance of alpha-fetoglobulins as a consequence of chemical carcinogenesis. Furthermore, it has direct applicability to prospective and follow-up studies of cancer patients. The test may ultimately prove useful in monitoring workers who are exposed to carcinogens. A radioimmunoassay for human α_1F has been available in our laboratory for over three years and is available for such use.

In addition, a major goal of the National Cancer Program is to develop techniques for the early detection of primary tumors prior to development of metastatic lesions. α_1F is the most obvious tumor related protein that might be used for this purpose. However, the tumors associated with α_1F production, hepatomas and teratocarcinomas, are very rare in the United States. Nonetheless, the feasibility of α_1F assays as a screening procedure is not completely irrational due to the potential ability to assay quantitatively large numbers of sera at little expense.

Proposed Course: The objective is to determine the biological significance of alpha$_1$ fetoprotein and other onco-fetal antigens, with regard to the relationship between the induction of primary hepatomas and the production of α_1F and other onco-fetal antigens. The major goal of the work will be directed toward determining the action of chemical carcinogens in vitro and in vivo in an attempt to explain how carcinogens, or other compounds such as phenobarbital, react with hepatocytes to initiate α_1F synthesis.

The biological function of α_1F, if any, remains only theoretical. Limited further studies will be directed toward understanding the biological function of α_1F. Considerable data related to synthesis, and catabolism during the growth of transplantable tumors, and during the feeding of chemical carcinogens has been collected and the biosynthesis of this protein in vitro has been studied.

Another objective is to develop immunoperoxidase and immunoelectron microscopic labelling techniques for α_1F and albumin in order to determine the cellular and subcellular distribution of α_1F in fetal liver, regenerating liver, hepatomas and carcinogen treated livers.

Finally, the identification, assay and significance of at least two other antigens will be studied, α_2 macroglobulin and the carcinogen induced preneoplastic antigen (PN) of Farber.

Date Contract Initiated: June 28, 1972

Current Annual Level: $241,000

CASE WESTERN RESERVE UNIVERSITY (NO1-CP-43354)

Title: Specific Immunological Unresponsiveness to Chemical Carcinogenesis and its Influence on Tumorigenesis

Contractor's Project Director: Dr. Jerome R. Pomeranz

Project Officer (NCI): Dr. Herbert Rapp

Objectives: To study the role of carcinogens which are contact sensitizing on the induction of tumors in sensitized or tolerant animals. Also, to study the role of adjuvants in suppressing the induction of mammary adenocarcinomas and other tumors following the infusion of the carcinogen.

Major Findings: Initially, methods to induce contact dermatitis and immune tolerance to 7,12, dimethylbenz[a]anthracene (DMBA) in guinea pigs were standardized. Preliminary studies suggest that guinea pigs sensitized with DMBA in complete freund's adjuvant (CFA) tend to be resistant to the tumorigenic action of DMBA irrespective of their immune state, while those sensitized topically developed tumors irrespective of tolerance or sensitization. However, the tumorigenic dose of DMBA used was toxic, and the surviving groups were small. In related studies, guinea pigs made tolerant by infusion of 5 to 20 mg. DMBA in Upjohn's fat emulsion in which sensitization was attempted with DMBA in CFA, did not develop tumors. A few infused animals, in which topical sensitization (without CFA) was attempted after an intravenous injection, developed adenocarcinomas of the breast. These results suggest that CFA delays primary breast tumor formation in guinea pigs following infusion of DMBA which may be comparable to the effects of BCG in other systems. Also, experiments showed that the depression effect of DMBA on the guinea pigs' ability to develop contact sensitization to picryl chloride persists for up to 6 weeks following the administration of DMBA.

It remains to be shown conclusively whether or not the recipients immune response to the carcinogen has an affect upon tumor induction by the same carcinogen. The development of breast carcinomas following the infusion of DMBA, and the finding that CFA delays primary tumor induction, requires further detailed investigation.

Significance to Biomedical Research and the Program of the Institute: The demonstration that guinea pigs may be contact sensitized and made tolerant provides a means of measuring the role of cell mediated immunity to these compounds. The effects of adjuvants on the suppression of carcinogen (DMBA) induced mammary adenocarcinoma in guinea pigs is being evaluated. This project will attempt to correlate the presence or absence of specific immunity to a carcinogen with the compound's capacity to induce a malignant tumor. Similarly, the possible role of adjuvants in suppressing primary mammary adenocarcinomas in guinea pigs is worth pursuing.

Proposed Course: Guinea pigs that are immunologically unresponsive or contact sensitized to DMBA have received a tumorigenic dose of DMBA

with minimum toxicity. Similarly, guinea pigs have been administered DMBA intravenously followed by adjuvant to study the induction as well as inhibition of mammary adenocarcinomas in guinea pigs.

Date Contract Initiated: February 15, 1972

Current Annual Level: $79,166

CHILDREN'S HOSPITAL MEDICAL CENTER (NO1-CP-45612)

Title: Effects of Carcinogens on the In Vitro Synthesis of Complement Components

Contractor's Project Director: Dr. Harvey R. Colten

Project Officer (NCI): Dr. Tibor Borsos

Objectives: The major goals of this contract consist of studies of the effects of selected chemical carcinogens, primarily, nitrosamines, on normal macrophage function. These macrophage functions include biosynthesis of the second (C2) and fourth (C4) components of complement, total protein synthesis, cell surface receptors and response to soluble mediators of macrophage mobility.

Major Findings: As noted previously administration of dimethylnitrosamine in vivo effectively inhibited biosynthesis of the second (C2) and fourth (C4) components of complement but this inhibition was masked by simultaneous administration of diphenylnitrosamine. In order to study the specificity of this masking effect the administration of DMN alone and in the presence of a turpentine induced sterile abscess were compared. An inflammatory reaction induced by turpentine was as effective as diphenylnitrosamine in stimulating C2 and C4 biosynthesis. The possibility was considered that DMN and diphenylnitrosamine might compete for a single enzyme thereby leading to neutralization of the DMN effect in a specific manner. Administration of DMN in the presence of a turpentine induced abscess suggests that a "nonspecific" inflammatory reaction may be equally effective. Similar results were obtained with dibutylnitrosamine (DBN).

Direct evidence that diethylnitrosamine (DEN) inhibits C2 and C4 biosynthesis was obtained in earlier studies and it seemed likely that this accounted for reduced serum levels of C4 in animals treated with DEN. However, an effect of DEN on C4 catabolism could not be ruled out. Studies of this aspect of C4 metabolism indicate that DEN has little if any effect on C4 catabolism. It may, in fact, slightly decrease the C4 catabolic rate. Thus the effect of DEN to reduce serum C4 is exclusively a result of depressed synthesis.

Studies of the effects of one carcinogen, DEN, on cellular immunity suggested that following establishment of delayed hypersensitivity, DEN had no significant effect on the expression or detection of the response.

Two experiments indicated that induction of the response might be blunted by previous exposure to DEN. One of these was a chance observation that exposure to BCG may be lethal in animals fed DEN.

Most of the experiments on the effect of DEN on humoral antibodies to sheep red blood cells (SRBC) suggested that this carcinogen had no effect on development of "natural" antibodies on the immune response to SRBC.

Finally studies of phagocytosis and chemotaxis indicate both a cellular and humoral defect which leads to impairment of both functions. These are independent of early complement components C1, C4 and C2. Thus the results indicate the need for a detailed examination of late acting complement components (C3-9) and the properdin proteins in carcinogen treated animals.

Significance to Biomedical Research and the Program of the Institute: This work provides insight into a number of basic alterations in effector cells of the immune system resulting from exposure to chemical carcinogens. In particular, preliminary findings suggest that several chemical carcinogens inhibit macrophage functions including complement biosynthesis, total protein synthesis, and membrane dependent macrophage activity. Some of the compounds are effective in vitro whereas others, such as DEN and DMN are only active in vivo. The project has significance for the control of human cancer inasmuch as the findings suggest a broader role of carcinogens in the etiology and pathogenesis of tumors. Hence, the major benefit of this program is in the area of defining the causes of cancer which, in turn, may lead directly to practical changes in therapy. Alterations in the immune capacity of experimental animals during administration of chemical carcinogens preceeded the appearance of frank tumor. Such changes, therefore, may be useful in monitoring the potential risk of tumor development and/or the efficiency of a particular mode of therapy. Extension of these studies to an analysis of basic aspects of cellular immune function in humans with cancer can be accomplished with well-defined techniques currently employed in a number of laboratories. The animal experimentation in this contract work permits a more rational choice of areas in immunology to study in a similar analysis of human tumors. Such a choice will maximize the probability of findings that will achieve the overall goals of cancer prevention and treatment.

In addition, the contract provides a relatively inexpensive, rapid way to examine the early events in carcinogenesis with particular emphasis on the effect of carcinogens on protein synthesis of immunologically active proteins.

Proposed Course: The findings of several effects of DEN and other nitrosamines on macrophage function in addition to its effect on complement synthesis require expansion of future work to include the following: (a) To identify additional quantitative and qualitative changes in macrophage function associated with in vivo administration of chemical carcinogens; (b) To determine whether these alterations in macrophage function affect immunologic competence during induction of chemical carcinogenesis;

(c) To examine alterations in complement and properdin proteins that affect phagocytosis and chemotaxis.

Date Contract Initiated: June 1, 1971

Current Annual Level: $73,778

DUKE UNIVERSITY MEDICAL CENTER (NO1-CP-33313)

Title: Response of Peripheral Blood Monocytes from Patients with Neoplastic Disease to Chemotactic Factors

Contractor's Project Director: Dr. Ralph Snyderman

Project Officer (NCI): Dr. Edward Leonard

Objectives: (1) To determine whether impaired delayed hypersensitivity in patients with cancer is related to deficient responsiveness of blood monocytes to chemotactic factors; (2) to determine if monocyte chemotaxis responsiveness is of prognostic significance in cancer patients; (3) to determine if chemotactic lymphokine production by peripheral blood lymphocytes from cancer patients is depressed; (4) to determine the effect of immunotherapy with BCG or C. Parvum and/or tumor removal on monocyte chemotactic responsiveness and lymphokine production in cancer patients; and (5) to determine the effect of chemical carcinogens or tumor implantation on macrophage chemotaxis in vivo and in vitro in mice.

Major Findings: 1. Monocyte chemotactic responsiveness is depressed in 54% of the 153 individuals with cancer studied thus far. Patients with depressed chemotaxis also had depressed delayed cutaneous hypersensitivity to ubiquitous antigens suggesting that depression of delayed hypersensitivity in cancer patients can result from defective monocyte chemotactic responsiveness.

2. Patients with locally recurrent malignant melanoma and depressed monocyte chemotaxis had a 50% incidence of distant metastasis or tumor related death within 4 months after initial testing. In contrast, patients with locally recurrent malignant melanoma and normal monocyte chemotaxis had only a 13% incidence of deep metastasis or death within the same time period. These findings suggest that depressed monocyte chemotaxis is associated with a guarded prognosis in patients with recurrent malignant melanoma.

3. Immunotherapy with BCG in patients with recurrent malignant melanoma and depressed chemotaxis enhances monocyte chemotactic responsiveness over at least a 2 month time period. It is not yet known if this enhancement of chemotaxis improves chances for patient survival.

4. Patients with depressed monocyte chemotaxis who undergo surgical removal of their tumors develop enhanced chemotactic responsiveness

shortly after tumor removal. These findings suggest that tumors themselves may depress monocyte chemotactic function and diminish the localization of macrophages at tumor sites.

5. Implantation of syngenic tumors in mice depress macrophage chemotaxis towards inflammatory stimuli in vivo and in vitro. These findings support the hypothesis that tumors may depress the ability of macrophages to accumulate at inflammatory sites and thus diminish the ability of a host to destroy its neoplasm.

Significance to Biomedical Research and the Program of the Institute: Evidence suggests that a function of the immune system is to protect the host against the development of neoplasms. By evaluating cellular immune effector function in patients with cancer it should be possible to determine if the inability of a host to destroy a neoplasm is due to a defect in the effector limb of the immune response. This information is relevant to an understanding of the mechanisms of tumor formation and subsequent development of methods for prevention, treatment, and possibly early diagnosis of cancer. The finding of depressed monocyte chemotactic responsiveness in patients with cancer and the ability of BCG immunotherapy to enhance chemotaxis in many patients may explain one mechanism by which BCG affords protection to cancer patients. Moreover, it will be determined if quantitation of monocyte chemotaxis provides a prognostic index for cancer patients and perhaps a guide for the effectiveness of various immunotherapeutic measures. The findings that tumors themselves might exert systemic deleterious effects on monocyte-macrophage chemotaxis is intriguing since this property of tumors might be a central means by which they escape immunologically-mediated destruction. We must now determine if patients with depressed chemotaxis have inhibitory factors in their serum or in their tumors. Isolation and characterization of such factors from humans and experimental animals will provide important information for our understanding of tumor development and provide new approaches for the therapy of cancer.

Proposed Course: The prognostic value and effects of immunotherapy or tumor removal on monocyte chemotactic responsiveness and lymphokine production will be determined. The mechanism(s) by which neoplasms depress immune effector function in humans and experimental animals will be explored. The effect of chemical carcinogens on immune effector functions in experimental animals will be studied to determine if depression of macrophage chemotaxis occurs prior to or subsequent to tumor development.

Date Contract Initiated: June 25, 1973

Current Annual Level: $75,769

HAMILTON BIOCHEMICAL RESEARCH LABORATORY (NO1-CP-45609)

Title: Supply P3 and Cell Walls from *Mycobacterium bovis* Strain BCG

Contractor's Project Director: Dr. Reno Parker

Project Officer (NCI): Dr. Herbert Rapp

Objectives: To prepare chromatographically pure P3 (trehalose mycolate) and cell walls from Mycobacterium bovis.

Major Findings: Using centrifugal chromatography, in combination with pressure elution chromatography approximately 360 mg of P3 (trehalose mycolate) has been produced at purity levels never before obtained. This material has shown considerable promise as an adjuvant in enhancing tumor regression.

Significance to Biomedical Research and the Program of the Institute: Effective prevention and treatment are proximate goals of the National Cancer Institute in its objective to control cancer in people. Investigations show that effective prevention can be obtained by use of BCG vaccine or its purified fractions. It is the objective of this contract to supply both cell walls and the glycolipid P3 from BCG so that determinations can be made relating to their ability to interfere with the carcinogenic process.

Proposed Course: To continue this service as long as deemed necessary by NCI.

Date Contract Initiated: June 24, 1974

Current Annual Level: $49,196

OHIO STATE UNIVERSITY (NO1-CP-53329)

Title: Chemical Carcinogenesis and Immunity

Contractor's Project Director: Dr. Bruce S. Zwilling

Project Officers (NCI): Dr. Virginia C. Dunkel
Dr. Tibor Borsos

Objectives: The major objective of this contract is to study the effects of non-tumorigenic levels of chemical carcinogens on normal immunological functions of Sprague-Dawley rats. This includes measurement of natural antibody levels to selected antigens, evaluation of the number and function of thymus-derived and bursal-derived lymphoid cells and comparison of macrophage function as measured by adherence, phagocytosis, glucose oxidation, response to lymphokines and tumoricidal and bactericidal activity. Additionally, tissues from treated and control populations will be examined histopathologically.

Major Findings: As this is a new contract, no major findings can be reported at this time.

Significance to Biomedical Research and the Program of the Institute: During the induction phase of chemical carcinogenesis the possibility is raised that in addition to an effect of an agent on the potential tumor cell, there is a concomitant effect of the agent on the immune capacity of the organism permitting establishment and growth of the aberrant cell type. This work will provide insight into a number of basic alterations in effector cells of the immune system resulting from persistant exposure to low levels of chemical carcinogens. The project has significance for human cancer in that the use of non-tumorigenic levels of carcinogen more closely approximate natural exposure of the human population to cancer causing agents. These studies may lead to a more definitive mode of cancer therapy based on fortification of the capacity to reject tumors by immunological mechanisms, as well as the identification of prognostic indicators associated with carcinogenesis and neoplastic transformation.

Proposed Course: This study will involve the systematic investigation of normal immunological functions, that is, those found in animals not obviously subjected to deliberate antigenic stimulation and concurrent measurement of these functions in carcinogen and analogue treated animals. Natural antibody levels to bacterial teichoic acid, E. coli, Salmonella typhosa and sheep red blood cells will be determined. Numbers and populations of thymus-derived cells will be measured by the presence and density of theta antigen; function will be assessed by T cell responsiveness to phytohemagglutinin and concanavilin A. Bursal-derived cells will be identified by the presence of surface immunoglobulin and the ability to form EAC rosettes. The response of these cells to lipopolysaccharide will also be evaluated. Macrophage activity will be determined by measuring adherence, phagocytosis of red blood cells, glucose oxidation, bactericidal and tumoricidal activity as well as response to lymphocyte supernatants. Histopathology will include complete blood counts and examination of all lymphoid organs and tumors.

Date Contract Initiated: March 25, 1975

Current Annual Level: $187,833

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CP-43276)

Title: In Vitro Study of the Nature of Interaction between Chemical and Viral Carcinogens

Contractor's Project Director: Dr. David Yohn

Project Officer (NCI): Dr. Joseph DiPaolo

Objectives: To determine whether known or potential chemical carcinogens as specified will cause morphologic and neoplastic transformation of selected human cells in vitro. To determine if these processes require potentiators such as hormones, DNA repair inhibitors or enzymatic activators of polycyclic carcinogens. To determine whether the selected known or

potential carcinogens enhance transformation of human cells by known tumor viruses SV_{40} and Feline sarcoma virus (FSV).

Major Findings: Alterations of growth characteristics including morphologic changes of WI-26 cells, of male human embryonic lung cells (HEL-M) and of Detroit-550 cells (human foreskin) has been obtained with BαP, 4NQO, MNNG, and occasionally with DMBA. Such cultures possessed increased cell density, greater doubling potential, loss of contact inhibition, increased cloning efficiency and ability to grow under or in soft agar. These effects were enhanced by the addition of 17-β-estradiol or cortisone acetate.

Transformation of Detroit-550 cells by SV_{40} was enhanced by pretreatment with BαP, MNNG, 4NQO and Acetoxy-AAF but not by MCA or DMBA. [Hydrocortisone and 17-β-estradiol enhance SV_{40} transformation in the presence of MNNG when the cells are pretreated with the hormones, mixed-function oxygenase activity in the cells can be induced with either BP or DMBA but not 3-MCA. Hydroxylated metabolites are formed and they bind to intercellular nucleophiles]. Transformation of Detroit-550 cells by Feline Sarcoma virus was enhanced by pretreatment with MNNG, 4NQO and Acetoxy-AAF.

Significance to Biomedical Research and the Program of the Institute: Although non-human mammalian cells have been malignantly transformed in vitro with known chemical carcinogens, there is no report of transformation of human cells by chemical carcinogens. It is of major importance to determine the conditions necessary to achieve neoplastic transformation of human cells by chemical carcinogens in vitro. An understanding of the process by which these carcinogens at least initiate the transformation process can be obtained by analyzing their ability to promote viral transformation of human cells.

Proposed Course: Since the present evidence indicates that certain carcinogens apparently at least initiate transformation (morphologic) of human cells, the major emphasis will be to augment or potentiate these changes. For example, Detroit-550 cells will be pretreated with MMS with and without hormones or DNA repair inhibitors prior to carcinogen treatment. Mixed-function oxidases will be added or induced in Detroit-550 cells prior to addition to polycyclic carcinogens. All transformants will be tested for their ability to form tumors in immunologic deficient hamsters or mice. Analysis of the enhancement and/or inhibition of SV_{40} transformation of human cells by carcinogens will be completed and similar studies with FSV initiated.

Date Contract Initiated: December 20, 1971

Current Annual Level: $131,849

PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK, INC. (NO1-CP-55679)

Title: Chemical Carcinogenesis and Immunity

Contractor's Project Director: Dr. Abraham G. Osler

Project Officers (NCI): Dr. Virginia C. Dunkel
Dr. Tibor Borsos

Objectives: The major objective of this contract is to evaluate several parameters of the normal immune mechanism during the process of chemical carcinogenesis. Apart from specific immune responses, host defenses are critically affected by the activities of the complement system. These will be assessed in experimental situations designed to simulate the oncogenic process in humans i.e., slowly-evolving malignancies which develop in a small percentage of an outbred population under the impact of low doses of the chemical carcinogen.

Major Findings: As this is a new contract, no major findings can be reported at this time.

Significance to Biomedical Research and the Program of the Institute: By far the greatest research effort on the immunology of neoplastic disease has dealt with the specific response of the host to his tumor burden. Moreover, these studies are generally carried out at a time when the tumor has already developed to a clinically-detectable stage. Little information is available on the events during the earliest stages of tumor cell growth. What is the immune status of the host during this period? Can the host marshall the nonspecific defense mechanisms available for limiting neoplastic cell growth? Does the developing tumor interfere with the host's capacity to mount an effective specific immune response? How are the varied functions of the complement system affected during the initial and later phases of tumor development? Information pertaining to these issues should provide the basis for a more rational approach to chemotherapy and specific immunotherapy, particularly if it should be found that the early carcinogenetic process is associated with significant alterations of the complement system - the major element in nonspecific host defense mechanisms and the intensifier of specific antibody functions.

Proposed Course: Sprague-Dawley rats, an outbred strain, will be fed with methylnitrosourea in several regimens designed to induce carcinogenesis in a small proportion of the animals. The sera of these rats, obtained at regular intervals, will be assayed for the overall activity of the classical and alternative complement pathways, as well as for their individual components. In addition natural antibody levels in the sera of tumor-bearing and control animals will be assayed during the entire period of observation to evaluate changes in this parameter that may be associated with chemical carcinogenesis. The animals fed with methylnitrosourea which do not develop neoplastic disease will also serve as an internal control in the evaluation of the effect of carcinogenesis on the immune status of the host.

Date Contract Initiated: March 25, 1975

Current Annual Level: $72,940

TEMPLE UNIVERSITY (NO1-CP-33200)

Title: Induction of Malignant Melanoma in Guinea Pigs

Contractor's Project Director: Dr. Wallace H. Clark, Jr.

Project Officer (NCI): Dr. Herbert Rapp

Objectives: To develop a model in a guinea pig which is analogous to one form of human cutaneous malignant melanoma; to determine whether or not this model can be altered by immunopotentiation with BCG or altered by immunosuppression with anti-lymphocyte globulin.

Major Findings: In an attempt to develop a model for malignant melanoma in the guinea pig, animals are being treated with DMBA, DMBA plus ultraviolet light, and DMBA plus ultraviolet light and psoralens.

Animals treated with DMBA show significant changes. At the sites of painting there is uniform hyperpigmentation, and within this hyperpigmentation there are four different kinds of lesions. The first of these lesions is a slightly elevated blue black nodule varying from less than 1 mm. to about 6-7 mm. in diameter. These nodules show a slight increase in the number of intra-epidermal melanocytes. The nodule is formed by a lesion in the dermis which consists of melanophages and melanocytes. Some melanocytes are slightly atypical but one cannot interpret this lesion as malignant melanoma. There is also a moderate increase in the number of intra-epidermal melanocytes throughout the painted area with striking prominence of dendrites containing large quantities of melanin.

At about 52 weeks small brown pigmented lesions developed within the painted areas. These may represent the early, true hyperplasia of melanocytes that possibly could be the beginning of the centrifugal growth phase of melanoma.

By the time 5 weeks has gone by, there is a striking increase in melanin synthesis in all painted animals suggesting that DMBA is a stimulator of melanin synthesis. In most human and animal systems, the only known mechanism for stimulation pigment synthesis is light. This carcinogen clearly induces pigment synthesis. It also apparently is not associated with pigment transfer from melanocytes to keratinocytes in any significant quantity. The induced synthesized pigment largely remains in the melanocytic system. This is in contrast to the effect of light. This phenomenon, if correct, may be of vital importance in studying melanogenesis and may be of importance in carcinogenesis, but this awaits the full development of this model.

It was also observed that the younger the animal is when the experiment starts the more likely these changes are to develop, and animals receiving DMBA in double dosage have lesions appearing 8-10 weeks earlier than animals receiving a single application of DMBA.

All of the animals that are beyond 52 weeks have apparently developed

definite keratinocytic lesions. These keratinocytic lesions may easily be interpreted as squamous cell carcinoma in-situ with a definite decrease in cellular cohesiveness. This squamous cell carcinoma is much closer to human squamous cell carcinoma than are lesions in mice.

Electron microscope studies revealed that there is a great increase in the amount of pigment synthesis with apparent block in pigment transfer due to DMBA. There are definite melanocytes in the dermis in the small blue nodules and an increased number of melanocytes. Abnormal melanin granules are being formed in some of the melanocytes.

At the fine structural level in the keratinocytic system, one of the earliest changes is abnormalities in the cell membrane with complex enfoldings and a decreased number of cellular attachments.

Significance to Biomedical Research and the Program of the Institute: The major goal of this project is to produce a tumor which is similar to human cutaneous malignant melanoma, with a biphasic growth pattern progressing to malignancy. If primary tumors with significant potential for biological malignancy evolve, the guinea pig model can be used for both immunotherapy and chemotherapy studies which have the possibility of direct applicability to man. An attempt to manipulate the evolution of a primary tumor that will progress to full biological malignancy through immunological means may block tumor progression and may illuminate the nature and behavior of malignant melanoma in man.

Proposed Course: (1) To continue these experiments until it can definitely be stated that a model of malignant melanoma analogous to man is or is not produced and to delineate the exact analogies in the exact places where this model differs; (2) To push both light and psoralens to the point where the time course and the yield can hopefully be accelerated: (3) After a model has been developed, to attempt to alter the model with immunoprotentiation and immunosuppression; and (4) To clearly delineate this as a model for squamous cell carcinoma of the skin in man.

Date Contract Initiated: October 1, 1972

Current Annual Level: $61,422

TEXAS, UNIVERSITY OF (at San Antonio) (NO1-CP-43353)

Title: Development of In Vitro Methods for the Detection of Cell-Mediated Immunologic Reactivity to Chemical Carcinogens

Contractor's Project Director: Dr. Daniel E. Thor

Project Officer (NCI): Dr. Herbert Rapp

Objectives: To determine the feasibility of devising in vitro immunologic tests for the detection of prior exposure to known chemical carcinogens.

Major Findings: To date three chemical carcinogens, producing immunological specific percutaneous reactions, are being evaluated; dimethylbenz[a]-anthracene (DMBA), methylcholanthrene (MCA), and benzpyrene (BP). These carcinogens behave as haptens and an antigenic structure that shows in vitro reactivity can be provided by a carcinogen-protein carrier complex. Two systems are being studied in parallel to couple carcinogens with carrier proteins. (1) Following sensitization by a modified split-adjuvant technique, the percutaneous application of C^{14}-labeled DMBA, MCA, or BP yields complexes that are formed in vivo and recovered by epidermal extractions. Multiple carriers bind carcinogen including albumin, transferrin, immunoglobulins & skin proteins to represent approximately 60% of protein bound carrier while to date 40% of carrier proteins remains unidentified. (2) In vitro complexes are obtained by an induced & isolated liver microsomal aryl hydrocarbon hydroxylase enzyme system. Multiple carcinogen-protein carrier conjugates are necessary in vitro to stimulate reactions. Using cavine whole serum for in vitro carrier-coupling, our data shows specific MIF indexes of 60±8 vs controls 90±9 for DMBA & only 78±7 for the promimal carcinogen, MCA in DMBA immunized guinea pigs.

Significance to Biomedical Research and the Program of the Institute: If successful, these studies will make it possible to determine whether human individuals have been exposed to a variety of agents known to be carcinogenic in animals and suspected of being carcinogenic in man. The development of an immunogenic carcinogen-carrier complex with specificity might also be very useful in surveillance studies. Significant differences among various populations in their immunologic reactivity to known carcinogens might make it possible to correlate the degree of reactivity with the subsequent development of cancer. Correlations of this kind might provide the impetus to remove the agents from the environment.

Proposed Course: Because of the multiple protein carriers that function as conjugates to complete antigen reactivity, feasible methods for human in vitro binding are now being devised. These carcinogen-carrier molecules will then be evaluated for specificity and compared to non-carcinogen molecules to establish base line date for human applications in the near future (next 2 years). Although MIF data appear to differentiate sensitized and nonsensitized animals, guinea pig blast transformation post-42-day immunizations appears to allow additional indices of lymphocyte reactivity and will be further evaluated.

Date Contract Initiated: February 16, 1972

Current Annual Level: $85,797

UTAH, UNIVERSITY OF (NO1-CP-45600)

Title: Carcinogenesis Bioassay Resource for Determining the Effect of Chronic Immunosuppression on Physical and Chemical Carcinogenesis

Contractor's Project Director: Dr. Ernst J. Eichwald

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: (1) To determine the effects of chronic treatment with methotrexate, ATS, ALS, cyclophosphamide plus ATS, hydrocortisone plus ALS, imuran, and prednisolone on benzpyrene and ultraviolet (UV) skin carcinogenesis on mice, and (2) to investigate the immunological aspects of UV carcinogenesis in mice.

Major Findings: (1) Dose and regime for methotrexate, ALS, and ATS which permit chronic administration without excessive toxicity have been determined. (2) Dosages and regimes for combined ATS plus cyclophosphamide, ALS plus hydrocortisone, imuran and prednisolone are currently under evaluation to determine effective, nontoxic immunosuppressive levels. (3) The effects of the immunosuppresive drugs on skin allograft rejection within and across the H-2 barrier have been investigated and are currently under further investigation using new preparations of ATS and ALS. (4) The appropriate dose of benzpyrene to use in the studies combining treatment with this carcinogen and immunosuppressive drugs has been defined. Recent experiments using fluorescent UV exposure have established the appropriate dosage to be used in the combined UV-immunosuppressive drug experiments. (5) Tumors induced by UV light have been determined to exhibit an unusually high degree of antigenicity, and are rejected in normal syngeneic mice. Tumor transplantation can be achieved, however, if the mice are immunosuppressed prior to tumor challenge (thymectomy, X-Ray, ALS, imuran, prednisolone, or methotrexate treated). (6) Transplants of UV tumors were successfully achieved in mice which were pretreated with UV irradiation alone (mercury lamp, 60sec/3x/wk) for as short a period as 3 weeks prior to tumor implantation. (7) Preliminary reconstitution experiments using equal numbers of lymphocytes (spleen and lymph node) from either aged matched controls or mice irradiated with UV for 3 months into thymectomized, X-irradiated recipients showed no difference in their ability to reject a UV tumor implant. (8) Preliminary experiments also suggest that UV irradiated animals cannot be reconstituted with lymphocytes from normal syngeneic donors. (9) UV tumor implants grew progressively in mice irradiated with UV light for 3 months and then removed from the UV treatment for 3 montsh. (10) Lymph nodes from chronically UV treated mice were observed to be much smaller than controls, possessing only 50% of the lymphocytes found in normal syngeneic animals. This suggests that the UV treatment in itself is somewhat immunosuppressive.

Significance to Biomedical Research and the Program of the Institute: This study is directed toward the following questions: Is the current trend toward increasing clinical use of immunosuppressive drugs likely to elevate the incidence of human malignant disease? Can the effects of these drugs on carcinogenesis be directly attributed to their immunosuppressive activity? Attempts are being made to provide information related to these questions through the use of carefully defined animal model systems. In addition, findings on the antigenicity of UV induced tumors provide a unique opportunity to investigate the mechanism which enables highly antigenic tumors to escape immunologic destruction in the primary host.

Proposed Course: (1) Proceed with studies combining treatment with carcinogens and immunosuppressive agents, (2) determine the immunosuppresive activity of the doses of drugs employed in the combination experiments in a variety of immunological test systems run concurrently with the combined treatment experiments, and (3) study immunologic parameters of UV carcinogenesis.

Date Contract Initiated: May 7, 1971

Current Annual Level: $271,830

SUMMARY REPORT

CARCINOGEN METABOLISM AND TOXICOLOGY SEGMENT

July 1, 1974 through June 30, 1975

The Carcinogen Metabolism and Toxicology Segment has as one of its goals the identification of those components of complex systems which function as environmental carcinogens and the development of analytical methodology for their detection and quantitation. Metabolic and toxicologic studies of environmental carcinogens in selected animal systems are conducted to provide additional knowledge on the metabolic pathways involved in the carcinogenic process. Such studies may also furnish an explanation for the varying responses of different animal species and man to chemical carcinogens. Mechanisms of chemical carcinogenesis in selected animal systems with defined characteristics are being conducted.

Nitrosamines Program

A primary concern of the Segment continues to be the development of a nitrosamines program with continued studies of their possible involvement in presumably spontaneous cancer in humans. Several contracts in this area are being supported by this Segment. A project at the Massachusetts Institute of Technology (NO1-CP-33315) is concerned primarily with the possible formation of nitrosamines in foodstuffs during preparation and storage or from materials consumed in the diet. The kinetics of the reactions of the food additives, sodium nitrite and nitrate, with food constituents to produce nitrosamines are investigated. Analyses of food and beverages from regions high in cancer incidence or where there is a high use of nitrate-containing fertilizer are continuing. The improvements in analytical methods for nitrosamines are also under investigation.

In related studies, at the Energy Research & Development Administration's Oak Ridge National Laboratory (YO1-CP-50200) the emphasis in the nitrosamine research is on the formation of simple dialkylnitrosamines from tertiary amines, especially drugs which are commonly used in this country. The conditions which favor or inhibit these reactions are being studied. The carcinogenic activities of certain nitrosamines and their structural analogs are being investigated. Minor changes in structure of some of the compounds can lead to very different effects in the carcinogenic activity or in the organotropic effect of the nitrosamine. Metabolism studies on some of the nitrosamines prepared at this laboratory are now being initiated.

A portion of the research supported at the Eppley Institute for Research in Cancer, University of Nebraska (NO1-CP-33278) is devoted to studies on nitrosamines. In this project the synthesis of various nitroso compounds, possible metabolites of carcinogenic nitroso compounds, and the testing of these substances for carcinogenic activity are being pursued. Studies on blocking agents are also conducted at the Eppley Institute: the naturally occurring substances, gallic acid and sulfite were found to block nitrosation reactions. Other phenolic compounds may also compete for nitrous acid thus reducing the

possibility of forming nitrosamines. Ascorbic acid can have a double effect in that it may block nitrosation reactions or it can enhance the carcinogenic action of preformed nitrosamines. Thus, the proposal that ascorbic acid should be added to nitrosatable drugs must be considered carefully. Biochemical studies on the effect of nitrosamines and their reactions with cellular constituent are continuing. It was found that the enzyme inducer pregnenolone 16α-carbonitrile inhibits the demethylation of dimethylnitrosamine. A project at the University of Mississippi (NO1-CP-43347) is largely concerned with the development and application of analytical methods for N-nitroso compounds: specifically, the concern is that many compounds which occur naturally in grain products have a secondary or tertiary amine structure and thus might be nitrosatable. However, during the past year it was shown by chemical studies that one of the most common of these cyclic amines could not be nitrosated by chemical means. This reduces the likelihood that such compounds would be nitrosated in vivo.

The projects at Oregon State University (NO1-CP-43348), British Food Manufacturing Industries Research (NO1-CP-44337), and Simon Frasier University (NO1-CP-43338) are concerned with the analysis of food products for nitroso compounds or their precursors. The contract with Thermo Electron Corp. (NO1-CP-45623) is concerned with the development of the thermo-luminous analyzer so that it can be used universally for detection of N-nitrosamines. If the preliminary results with the thermo-luminous analyzer are borne out, this instrument would obviate the need for extensive clean-up procedures in analysis of foods and other environmental materials for nitrosamines. The sensitivity of this instrument is also much greater than any of the other techniques in use at present.

Mechanistic and Metabolic Studies

With regard to the metabolism of carcinogens, the Segment is supporting two projects at the Massachusetts Institute of Technology. One of these (NO1-CP-43265) is concerned with aflatoxin and other mycotoxins which have been identified in foodstuffs from Southeast Asia. The metabolism of aflatoxin in vivo and in vitro, its biochemical effects, and the identification of the metabolites from various species are being studied. In addition, the identification and possible carcinogenic activity of other mycotoxins from foodstuffs are being investigated. Another project (NO1-CP-33238) is studying the effects of lipotropes in chemical carcinogenesis. At present these efforts emphasize largely the effects of lipotropes on colon carcinogens due to the high incidence of colon cancer in much of the western world. A definitive mechanism for their action has not been elucidated, however. The Segment had also supported a project at Temple University (NO1-CP-51029) on the possible occurrence of carcinogenic mycotoxins in dermatophytes which are commonly found in humans. The final report showed that there was no apparent carcinogenic effect from the various fractions isolated under the conditions used.

The induction of lung adenomas in strain A mice is being used to test various alkylating agents, drugs, alkyl halides and other environmental factors at the University of California at San Diego (NO1-CP-33232). Within its limitations this technique furnished a relatively faster method for screening of

presumed carcinogens. Studies on the combined effects of various known oncogenic viruses and chemical carcinogens or inhibiting effects on chemical carcinogens are being followed. As part of a new project the Segment will support a study on the metabolism of certain other environmental carcinogens such as dye-stuff or polymer intermediates and fumigants.

Mutagenesis Prescreening Program

The Segment is also concerned with the evaluation of mutagenicity tests as methods for prescreening compounds for possible carcinogenic activity. There is evidence that many compounds which are mutagenic are also carcinogenic and that many carcinogens are also mutagenic, especially in the activated form. Several contracts are being supported presently to determine the correlation between the carcinogenicity and mutagenicity of approximately 100 compounds (known carcinogens of various types and non-carcinogenic analogs) in various bacterial and mammalian cell systems, both with and without an activation system (Stanford Research Institute [NO1-CP-33394], Columbia University [NO1-CP-33395], and Arthur D. Little, Inc. [NO1-CP-33392]). The same compounds are also being studied at Stanford Research Institute in a DNA repair system. Some of the preliminary results from the bacterial mutagenicity studies are quite promising in that there appears to be a good correlation between carcinogenic and mutagenic activity. The results with the mouse lymphoma cell systems are less promising. It appears that the mammalian cell systems need further development before they could be used as a prescreening method.

CONTRACT NARRATIVES

CARCINOGEN METABOLISM AND TOXICOLOGY SEGMENT

July 1, 1974 through June 30, 1975

ARTHUR D. LITTLE, INC. (NO1-CP-33392)

Title: Use of Lymphoma Cells In Vitro and in Host-Mediated Bioassays as a Potential Prescreen for Chemical Carcinogens

Contractor's Project Director: Dr. Jerry J. Carter

Project Officer (NCI): Dr. Lionel Poirier

Objective: The objective of this project is to study the mutagenic activity of chemicals in L5178Y cells using resistance to methotrexate (MTX), thymidine (TdR), and cytosine arabinoside (Ara C) as genetic markers. The relationship of dose response to mutagenic activity will be evaluated for a selected number of compounds in vitro and without metabolic activation.

Major Findings: L5178Y lymphoma cells have been used for the in vitro and host-mediated assay systems to assay chemicals for mutagenic activity. These cells show high cloning efficiency in culture when removed from the host. The system employs resistance to three nucleic acid inhibitors as markers (cytosine arabinoside, methotrexate and excess thymidine) to demonstrate mutagenicity. Nonmutant (sensitive) cells do not grow in the presence of the inhibitors, permitting growth of the mutant clones.

Chemicals tested in vitro included 3 carcinogens, 10 noncarcinogens, 30 procarcinogens, 1 promoter, and 14 ultimate carcinogens. Presumptive mutagenic activity was detected in each category at one or more genetic loci. Although 90% of the carcinogens showed mutagenic activity, 80% of the non-carcinogens were also mutagenic. Modification of technical and statistical methodologies are now in progress which should make the system more discriminatory while retaining the current sensitivity for detecting carcinogens. The methotrexate and thymidine markers appear to be most sensitive to the action of the agents studied to date. The dose response data varied. Smooth dose response increases were found for ethylmethane sulfonate at the TdR marker and for succinic anhydride at the Ara C and TdR markers. More frequently, dose responses were nonlinear with the largest increments of mutants occurring at the lower concentrations of the chemicals. Twenty compounds were tested in the in vivo assay system.

Significance to Biomedical Research and the Program of the Institute: The L5178Y system as currently used in vitro (and to some extent, in vivo) detects the mutagenic activity of significant proportions of procarcinogens and carcinogens. With some modifications, it may prove to be an important assay to reduce the number of hazardous chemicals in the human environment. It is capable of incorporating a number of additional parameters which can enhance its versatility in detecting mutagenic activity which might be correlated with carcinogenic activity.

Proposed Courses: Multiple in-depth studies of a number of chemicals testing each marker are in progress in order to obtain additional information as to whether (1) differential growth rate occurs for mutant and sensitive cells, (2) there are selective effects between sensitive and resistant cells in mixed populations, and (3) the expression time is the same for each marker in repeated tests. These plus additional studies in vitro and in vivo to increase marker discrimination between activity of noncarcinogens and carcinogens are also proposed.

Date Contract Initiated: June 19, 1973

Current Annual Level: $144,047 (for nine months)

BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH (NO1-CP-43337)

Title: Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment

Contractor's Project Director: Dr. C. L. Walters

Project Officer (NCI): Dr. Larry K. Keefer

Objectives: To develop a method for the determination of non-volatile nitrosamines directly on solid phase samples without the necessity for extraction and to determine its scope and limitations on application.

Major Findings: The agent found to be most effective for the release of volatile denitrosation products from N-nitroso derivatives dissolved or suspended in a non-polar solvent is oxalyl chloride. On trapping the main product, nitrosyl chloride, in alkali, it forms not only nitrite but also some nitrate and nitric oxide; use is therefore being made of alkaline hydrogen peroxide as a trapping system, followed by the determination of the products of denitrosation as nitrate.

Significance to Biomedical Research and the Program of the Institute: Nitrate is widely distributed in the environment and is readily reduced to nitrite. Some nitrosatable amines present in biological systems can yield N-nitroso derivatives which can be separated on the basis of their volatility or extractability while other such derivatives are extracted with extreme difficulty, if at all. Most N-nitroso derivatives are carcinogenic or can lead to such carcinogens by transnitrosation; thus, a method of determination of non-volatile compounds of this type would permit epidemiological studies on the basis of the exposure of different populations, in accordance with the objective of the Institute in pinpointing any relationship between environmental agents and cancer in man.

Proposed Course: The development will be continued of the method of determination of total non-volatile nitrosamines at the µg per kg level using a procedure involving their denitrosation in situ on a biological matrix without prior extraction. The response will also be ascertained from compounds such as S-nitrosothiols which could arise from nitrate or nitrite

in a biological matrix. The technique of choice will be applied to freeze-dried foods and other environmental samples of concern in cancer epidemiology.

Date Contract Intitiated: June 1, 1974

Current Annual Level: $27,270

CALIFORNIA, UNIVERSITY OF (SAN DIEGO) (NO1-CP-33232)

Title: Pulmonary Tumors in Mice for Carcinogenic and Co-Carcinogenic Bioassay

Contractor's Project Directors: Dr. Michael B. Shimkin
Dr. Alexis J. Kniazeff

Project Officer (NCI): Dr. Elizabeth K. Weisburger

Objectives: The objectives of this project are twofold: (1) to test environmental compounds for carcinogenic activity using the strain A mouse pulmonary-tumor-induction-technique and (2) to evaluate the effect of respiratory virus infections on the chemical induction of pulmonary tumors in strain A mice.

Major Findings: (1) Bioassay - The strain A mouse lung tumor system was applied to the bioassay of 15 food additives from the GRAS list, 10 silylating agents, 15 metals, 2 chemotherapeutic drugs, 5 solvents, 2 flameproofing agents, and 2 synthetic ripening chemicals for tobacco and fruits. The silylating agents are used in gas chromatography to convert nonvolatile compounds to volatile materials for GC determinations. The flameproofing agents are used in the manufacture of children's sleepwear. The tumor response of chemically-treated mice was compared to that of the appropriate controls with the following results:

(a) Food Additives - At the dose levels employed, all food additives were negative for lung tumor production in A mice.

(b) Silylating Agents - 1,3-bis(chloromethyl) 1,1,3,3-tretamethyl-disilazane, N,O-bis (trimethylsilyl)acetamide (BSA), 1-ethyl-3-p-tolyltriazene, N-acetylimidazole, N-trimethylsilylimidazole, and trimethylchlorosilane, were carcinogenic in mice under the conditions employed. Hexamethyldisilazane (HMDS), N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA), N-trimethyl-silylacetamide, and tert-butyldimethylchlorosilane were negative at the dose levels employed.

(c) Metals - Bioassays have been completed on five of the fifteen metals. The five metals; viz, calcium, cobalt, lead, cadmium and manganese, were negative for lung tumor production in A mice.

(d) Data have not as yet been obtained from bioassays of the chemotherapeutic drugs, solvents, flameproofing agents, and the synthetic ripening chemicals.

(2) Virus-chemical Carcinogenesis - Data reported previously showed that experimental infection of strain A mice with the Moloney strain of murine sarcoma virus caused a 30-50% reduction in the lung adenoma response to 3-methylcholanthrene and urethan. During the past two years, these studies were extended to include certain non-oncogenic viruses. It has been demonstrated that infection of A mice with murine Reovirus administered by inhalation results in a similar reduction in the adenoma response to urethan. Repeat studies are being conducted to confirm these results and to determine the effect(s) of various schedules of Reovirus-urethan administration on the adenoma response.

Significance to Biomedical Research: This project will integrate into the activities of the Carcinogenesis Program by attempting to develop a rapid and reliable bioassay of chemicals for carcinogenic activity. Research in this area is of high priority due to the vast expense and time required to screen chemicals by the presently used animal bioassays. This research project will also provide timely extension of the existing bioassay capabilities.

Proposed Course: To continue work in the areas outlined above.

Date Contract Initiated: June 22, 1970

Current Annual Level: $143,645

COLUMBIA UNIVERSITY (NO1-CP-33395)

Title: In Vitro and Host-Mediated Microbial Mutagenicity as a Prescreen for Chemical Carcinogens

Contractor's Project Director: Dr. Herbert Rosenkranz

Project Officer (NCI): Dr. Lionel Poirier

Objectives: The objective of this project is to determine whether microbial assays to test mutagenicity and DNA-modifying ability could be used to detect potential carcinogens. If so, then this system will eventually be one in a battery of mutagenicity assays used as prescreens of environmental chemicals for carcinogenicity.

Major Findings: Over 100 chemicals comprising non-carcinogens, ultimate carcinogens and proximate carcinogens have been tested for their ability to preferentially inhibit the growth of DNA polymerase-deficient Escherichia coli (E. coli pol A_1^-) and to induce mutations to histidine-independence in a series of Salmonella typhimurium tester strains. All tests were carried out both in the presence and in the absence of a microsomal activation mixture.

(1) It was found that the Salmonella typhimurium tester strains exhibited unexpected phenotypes as a result of the genetic deletion introduced to increase their permeability to large molecules. The effect of these phenotypes on the response to mutagens and potential carcinogens was evaluated.

(2) It was discovered that visible light (450 nm) was mutagenic for some of the Salmonella tester strains. This finding is an indication that the various manipulations should be carried out in subdued light and that incubations should be carried out in the dark.

(3) A number of modifications of the "standard" assay procedures were introduced which increased the usefulness of these bioassays.

(4) In spite of improvements of the procedures some carcinogens give positive results only in one or the other of these bioassays which suggests that in routine screening both procedures be used in tandem.

(5) Using the two microbial assays in tandem, approximately 82-86% of known carcinogens give positive tests.

(6) These assays were applied to a study of the urines of patients receiving various forms of medications. It was found that the assays can be readily adapted for screening human urines. Moreover it was found that the urines of patients receiving certain therapeutic agents contained substances (presumably metabolites) which gave positive tests.

Significance to Biomedical Research and the Program of the Institute: It appears that microbial assays are ready for use in a battery of tests designed to detect environmental carcinogens. The procedures are rapid and inexpensive and if adopted may be extremely useful in detecting and identifying environmental carcinogens. Removal of such agents from the environment may result in an appreciable decrease in the incidence of cancers due to environmental agents.

Proposed Course: (1) To determine dose-response curves for some of the agents which are positive in these microbial assays. This in turn may reveal certain structure-activity relationships, and (2) to improve these bioassays further so as to broaden their usefulness for the detection of potential carcinogens in the environment and in biological fluids.

Date Contract Initiated: June 18, 1973

Current Annual Level: $77,330 (for nine months)

ERDA-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) (Y01-CP-50200)

Title: NCI-ERDA Carcinogenesis Program

Contractor's Project Director: Dr. Francis T. Kenney

Project Officer (NCI): Dr. Allen Heim

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NIH-NO1-CP-43265)

Title: Toxicity and Carcinogenicity Associated with Fungal Growth on Foodstuffs

Contractor's Project Directors: Dr. Gerald N. Wogan
Dr. George H. Büchi
Dr. Arnold L. Demain

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: To investigate the biochemical, toxic, and carcinogenic effects of aflatoxins, their metabolites and analogs; to determine the metabolic fate of aflatoxins in various species; to synthesize aflatoxin metabolites; and to isolate, characterize, and investigate the toxicity and carcinogenicity of other mycotoxins produced by molds isolated from foods or food raw materials.

Major Findings: Toxicity and carcinogenicity evaluation of aflatoxins M_1, P_1, and Q_1 are in progress. Metabolism of aflatoxin B_1 by microsomal preparations of livers of various animal species and man is being studied in vitro. Isolates of toxin-producing fungi previously collected from foods in Thailand were cultured on various media to provide material from which new mycotoxins can be isolated. Three new compounds have been isolated during the current year: two are tremorigenic agents produced by A. clavatus for which structures are established; three are highly toxic metabolites of Chaetomium mollicellum, Ames; and one a cyclic pentapeptide, produced by A. niger, related in structure to malformins.

Significance to Biomedical Research and the Program of the Institute: Evidence is accumulating that chemical products arising from microbial spoilage (particularly involving mold-damage) of foods or food raw materials can appear in the human food supply and may be associated with the etiology of liver cancer in several areas of Asia and Africa. The scope of the problem in terms of numbers of compounds and/or molds that may be involved has not yet been accurately defined, but is potentially great. This project, which is related to other NCI contracts on naturally-occurring carcinogens, is designed to produce relevant information on these questions.

Proposed Course: Experiments will continue along the lines suggested by the objectives outlined above. Studies on the comparative metabolism of aflatoxins may help to delineate the mechanisms involved in rather wide species differences in response to these compounds. Similarly, studies on the biochemical effects, toxicity and carcinogenicity of analogs and metabolites may provide evidence on active forms of the agent, and on sites of action. Identification of new mycotoxins from other molds isolated from food samples will provide further definition of the scope and character of the potential hazards to man represented by these toxins.

Date Contract Initiated: June 25, 1962

Current Annual Level: $218,480

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CP-33238)

Title: Interactions Between Diet and Chemical Carcinogenesis: A Bioassay System

Contractor's Project Director: Dr. Adrianne E. Rogers

Project Officer (NCI): Dr. Lionel Poirier

Objectives: To develop in rats a bioassay model for carcinogens using lipotrope deficiency which reproduces nutritional conditions and effects under which people may be exposed.

Major Findings: A semisynthetic diet, which provides adequate levels of nutrients for growth of rats but is marginally deficient in the lipotropes choline, methionine and folic acid, (1) enhances induction of liver tumors by 5 of 6 carcinogens tested, (2) enhances induction of colon tumors by sym-dimethylhydrazine, (3) does not affect induction of tumors of the stomach or urinary bladder, (4) decreases induction of mammary tumors by AAF but not by DMBA, and (5) decreases hepatic microsomal oxidases and clearance of DEN.

Significance to Biomedical Research and the Program of the Institute: Dietary effects on chemical carcinogenesis can be demonstrated in experimental animals and are strongly indicated by epidemiologic studies in humans. Development of bioassay systems in animals utilizing known dietary effects will (1) permit definition of the dietary components which may retard or enhance chemical carcinogenesis, (2) provide models in which study of metabolism of carcinogens and other mechanisms of carcinogenesis can be made and related to differences in effective carcinogenesis, and (3) provide a sensitive system for testing compounds which give equivocal results in normal rats.

Proposed Course: (1) Completion of experiments comparing tumor induction in normal or lipotrope-deficient rats by carcinogens of different chemical classes and affecting different target organs, (2) definition of the specific dietary components responsible for the enhancement of carcinogenesis by the lipotrope-deficient diet, and (3) measurement of the effect of the deficient diet on (a) urinary metabolites of AAF; (b) hepatic sulfhydryl compounds; and (c) metabolites of DEN in the peripheral blood.

Date Contract Initiated: February 1, 1972

Current Annual Level: $126,755

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CP-33315)

Title: Environmental Occurrence of N-Nitroso Compounds

Contractor's Project Directors: Dr. Steven R. Tannenbaum
Dr. Michael C. Archer

Project Officers (NCI): Dr. Elizabeth Weisburger
Dr. Larry Keefer

Objectives: The objectives of this contract are (1) to develop analytical procedures for the isolation, detection, and quantitation of N-nitroso compounds in foods and beverages; (2) to examine foods and beverages for N-nitroso compounds and their precursors in cancer-rich areas; (3) to define conditions which favor the formation of nitrosamines and conditions which inhibit their formation; (4) to determine the toxicity of newly discovered N-nitroso compounds; and (5) to investigate the formation of nitrite and nitrosamines from their precursors in the oral cavity.

Major Findings: High-performance liquid chromatography has been used for separation of non-volatile nitrosamines. A new liquid chromatography detector for nitrosamines has been developed employing continuous photolytic decomposition of the nitrosamine followed by quantitation of the nitrite so produced by a continuous colorimetric procedure. Preliminary analysis of drinking water, saliva, and urine from a region of Colombia where there is a high risk of stomach cancer has revealed that there may be a correlation between high urinary nitrate and salivary nitrite levels and cancer risk. The presence of cationic micelles has been shown to increase the rate of formation of certain nitrosamines by several orders of magnitude. A series of carbonyl compounds has been shown to catalyze the formation of nitrosamines over a wide pH range. Nitrosamine formation has been demonstrated in human saliva using morpholine, diethylamine and dimethylamine as the precursor amines. Rates of formation are too high to be explained by simple chemical reaction with nitrite, and microbial catalysis is implicated.

Significance to Biomedical Research and the Program of the Institute: The carcinogenicity of large numbers of nitrosamines has been demonstrated in various tissues of many animal species. These compounds which can form in the environment could represent a health hazard and may be relevant to the etiology of human cancer. This project will aid in determining the total extent to which nitrosamines may be present in the environment and conditions which favor or retard their formation and degradation in foods and beverages during their processing, storage, and consumption.

Proposed Course: The project will continue in the areas outlined above. Analytical techniques will be developed for the non-volatile nitrosamines. Samples from geographical regions having a high incidence of cancer will be examined for nitrosamines and their precursors. New factors will be investigated which affect the rate of formation of nitrosamines. Research will continue on nitrite and nitrosamine formation in human saliva.

Date Contract Initiated: June 11, 1970

Current Annual Level: $132,343

MISSISSIPPI, UNIVERSITY OF (SCHOOL OF PHARMACY) (NO1-CP-43347)

Title: Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment

Contractor's Project Director: Dr. John K. Baker

Project Officer (NCI): Dr. Larry Keefer

Objectives: (1) To prepare 25 g samples of several benzoxazolinones, their N-hydroxy derivatives, and their N-nitroso derivatives for carcinogenesis screening and (2) to develop analytical methods for the N-nitroso derivatives of benzoxazolinones and related non-volatile or unstable nitrosamines in foodstuffs such as corn, wheat, and rye.

Major Findings: N-Nitrosation of benzoxazolinones in grain products does not readily occur. However, the total N-nitroso content of grain products was found to be as high as 500 ppb. The identities of these compounds are not known at this time.

Significance to Biomedical Research and the Program of the Institute: New strains of corn and other grain products have been introduced into commerce that have high benzoxazolinone content. Through the use of nitrate fertilizers, these compounds may be converted to the potentially carcinogenic N-nitroso derivatives. Analytical methods for individual thermally unstable, nonvolatile N-nitroso compounds do not exist at this time. Under this contract, these methods are being developed and applied to food samples. Present information indicates this class of compounds may be present in larger quantities than the volatile nitroso compounds.

Proposed Course: Continue the development of high pressure liquid chromatographic procedures for N-nitroso standards presently available.

Date Contract Initiated: May 1, 1974

Current Annual Level: $17,209

OREGON STATE UNIVERSITY (NO1-CP-43348)

Title: Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment

Contractor's Project Director: Dr. Donald D. Bills

Project Officer (NCI): Dr. Larry Keefer

Objectives: (1) To analyze foodstuffs or environmental samples for individual N-nitroso compounds and their precursors using established methods, (2) to examine new analytical techniques which may become available, and (3) to conduct other research aimed at understanding the formation, identification, quantitation and destruction of N-nitroso compounds in the human environment.

Major Findings: The following products were found not to contain volatile nitrosamines (minimum level of analysis 5 ppb): local mushrooms (fresh and canned), kippered herring (Norway), smoked clams (Japan), canned clams (domestic), cheeses (imported Gouda, Tilsit, Danbo; domestic Kraft processed). Thirteen samples of cooked bacon contained an average level of approximately

15 ppb nitrosopyrrolidine in the combined bacon and cooked-out fat. An additional 5 ppb (average) was volatilized during the cooking process and trapped from the vapor. A series of pyrazines which could interfere with nitrosamine analysis were identified in cooked bacon. Nitrosohydroxyproline heated under conditions to simulate bacon frying yielded nitrosohydroxypyrollidine as the major Griess-positive product.

Significance to Biomedical Research and the Program of the Institute: Until the qualitative and quantitative aspects of the occurrence of N-nitroso compounds and their precursors in the human environment have been more thoroughly documented, it will not be possible to determine human exposure and, consequently, the potential significance to human health. The significance of the contract lies in the accumulation of knowledge of the distribution of N-nitroso compounds and their precursors in the environment. Concurrently, the investigation of new or improved analytical techniques for the analysis of N-nitroso compounds should improve the analytical state of the art here and elsewhere. Finally, it is important, as time allows, to understand more completely the mechanisms by which N-nitroso compounds are formed and destroyed in the environment.

Proposed Course: To continue analyzing samples of interest and to further investigate nitrosohydroxyproline as a precursor of potential carcinogens and the role of collagen in the formation of N-nitroso compounds during the cooking of bacon.

Date Contract Initiated: May 2, 1974

Current Annual Level: $54,020

SIMON FRASER UNIVERSITY (NO1-CP-43338)

Title: Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment

Contractor's Project Director: Dr. Y. L. Chow

Project Officer (NCI): Dr. Larry Keefer

Objective: To critically review the existing knowledge on photolysis of nitrosamines and evaluate its applicability for analyses of nitrosamines in the human environment and to refine available methods for photochemical analysis of nitrosamines in environmental samples.

Major Findings: It was established that photohydrolysis of N-nitrosodimehylamine (NND) in a basic aqueous solution by $\pi \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$ excitation does not give a quantitative yield of nitrous acid as has been claimed. Oxidative photolysis ($\pi \rightarrow \pi^*$ excitation) of NND in the presence of tetrahydrofuran followed by a basic decomposition gave 60% of nitrite ion which was determined by the Griess method. The same analytical procedure in the presence of isopropyl alcohol gave nitrate ion and acetone in a nearly quantitative yield; however, it was found that nitrate ion could not be determined

quantitatively by the reductive Griess method.

Significance to Biomedical Research and the Program of the Institute: It is hoped that photochemical decomposition of nitrosamines as described above can be adapted for analysis of these carcinogens in environmental samples without extensive extraction processes.

Proposed Course: It is proposed to use other hydrogen donor substrates, such as trioxane and piperonal, in order to achieve the quantitative yield of nitrite ion. Alternatively photohydrolysis of nitrosamines in the presence of other nucleophiles, such as azide and cyanide ions, will be investigated.

Date Contract Initiated: June 1, 1974

Current Annual Level: $11,960

STANFORD RESEARCH INSTITUTE (NO1-CP-33394)

Title: Potential Prescreens for Chemical Carcinogens

Contractor's Project Directors: Dr. Vincent F. Simmon
Dr. Ann D. Mitchell

Project Officer (NCI): Dr. Lionel Poirier

Objectives: Microbial mutagenicity and the detection of unscheduled DNA synthesis in human tissue culture cells have been proposed as rapid, sensitive, and economical prescreening techniques to identify chemical carcinogens. The objectives of this research are to develop and test these systems using 103 chemicals known to be ultimate carcinogens, procarcinogens, or noncarcinogens.

Major Findings: (1) Microbial mutation - Approximately 80% of the carcinogens tested have been detected as mutagens by a combination of the Salmonella typhimurium assays and assays with Saccharomyces cerevisiae D3. Salmonella typhimurium strain TA1536 has been found to be of little value in detecting many carcinogens as mutagens and therefore is no longer being used. The host-mediated assay has not been as reliable an indicator of carcinogens as has been thought. (2) Unscheduled DNA Synthesis - Autoradiography was used initially to measure DNA repair in contact-inhibited diploid human fibroblasts, WI-38 cells, following in vitro exposure to 25 representative ultimate carcinogens, procarcinogens, or noncarcinogens. The chemicals were tested in the presence and in the absence of a metabolic activation system. It was found that metabolic activation reduced the level of repair following exposure to ultimate carcinogens and increased the level of repair following exposure to procarcinogens. Significant levels of repair were not observed following exposure of the cells to noncarcinogens---either with or without metabolic activation. As the autoradiography approach seemed relatively inefficient and time-consuming for testing each chemical over a wide range of concentrations, a modified assay system was developed in which UDS is measured by scintillation counting. The scintillation counting approach has proven to be

sensitive and efficient. The results of each assay, using a wider range of concentrations, can be obtained within 24 hours, and over a 95 percent correlation of the UDS results with the results of in vivo bioassays has been observed for the compounds tested to date.

Significance to Biomedical Research and the Program of the Institute: A vast majority of cancers appear to be caused by chemical or physical agents, and modern technology is continually increasing the number of such potentially hazardous agents which invade man's environment. As rapid in vitro testing procedures, the microbial mutation and unscheduled DNA synthesis assays should be of vital importance in prescreening large numbers of these substances.

Proposed Course: It is proposed that the testing which has been described will be continued and further utilized in determining basis of carcinogenesis and in pursuing the goal of cancer prevention.

Date Contract Initiated: June 3, 1973

Current Annual Level: $159,095 (for six months)

THERMO ELECTRON CORPORATION (NO1-CP-45623)

Title: Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment

Contractor's Project Director: Dr. David H. Fine

Project Officer (NCI): Dr. Larry Keefer

Objectives: Development and application of new methods for the sub-ppb analysis of both volatile and non-volatile N-nitroso compounds in the environment.

Major Findings: The N-nitroso compound specific Thermal Energy Analyzer has been interfaced to both a gas chromatograph (gc-TEA), and a high performance liquid chromatograph (hplc-TEA). Using the gc-TEA, rapid analytical procedures sensitive at the 0.002 µg/kg level for gc amenable N-nitroso compounds have been developed. The hplc-TEA technique is not yet fully developed, nevertheless it can now be used for the selective analysis of non-volatile N-nitroso compounds in nanogram amounts.

Significance to Biomedical Research and the Program of the Institute: Sensitivity at the 0.002 µg/kg level is needed for metabolic studies in small laboratory animals and also for evaluating the in vivo distribution of N-nitroso compounds. Because the time per analysis has been reduced from days to hours, the cost per analysis has been greatly reduced.

Currently, adequate analytical methods or techniques are not available for the majority of the non-volatile N-nitroso compounds. Using the hplc-TEA, adequate methods can now be developed.

Proposed Course: In addition to further developing the analytical procedures, other scientific studies aimed at understanding the environmental distribution of N-nitroso compounds will be carried out.

Date Contract Initiated: June 18, 1974

Current Annual Level: $76,023

SUMMARY REPORT

CHEMISTRY AND MOLECULAR CARCINOGENESIS SEGMENT

July 1, 1974 through June 30, 1975

The major objective of the Chemistry and Molecular Carcinogenesis Segment is to gain an understanding of the molecular process involved in chemical carcinogenesis and an understanding in chemical and biological terms of the important events that lead to carcinogenesis. These studies have been grouped into the following five major approach areas: (1) biochemical properties of chromatin and nucleic acids; (2) study of surface membranes and the manner in which they participate in the regulation of cellular properties; (3) study of polycyclic hydrocarbons and the significance to carcinogenesis of the many products which result from their enzymatic activation; (4) study of the genetics of somatic cells, the chemically-induced mutations that can be induced in them, and the significance of this process in carcinogenesis; and (5) genetic studies in human populations.

Biochemical Properties of Chromatin and Nucleic Acids

Fundamental research has continued at the Energy and Research Development Administration's Oak Ridge National Laboratory (Y01-CP-50200) into the mechanisms of DNA repair in mammalian cells and the role of UV-induced pyrimidine dimers (in DNA) in the malignant transformation of cells induced by UV light. It has been shown that carcinogens generally fall into two classes (UV-like and X-ray-like) in terms of the repairability of DNA lesions induced by these carcinogens in various cell lines. Normal human cells can excise essentially all UV-induced pyrimidine dimers if the dose of UV is small enough. Recent work has indicated that the formation of pyrimidine dimers in DNA is an initial alteration capable of inducing malignant transformation. This finding is of particular interest as it illustrates in a specific fashion the role of the genome in the development of cancer.

Investigations at the University of Texas (NO1-CP-43270) have led to reproducible techniques for isolating non-histone, DNA-binding proteins from normal rat liver and chemically-induced rat hepatoma. This class of proteins is thought to be regulatory. These proteins have been characterized with respect to molecular weight, ability to restrict chromatin-templated RNA synthesis, lack of phosphorylation in vivo, the specificity of binding to homologous DNA, and tissue-of-origin specificity as determined by immunochemical characteristics. On treatment with hepatocarcinogens, the properties of the isolated proteins change from normal to those characteristic of hepatoma. Whether the change in specificity is a function of normal/malignant properties or is due to concomitant changes in cell type is under study.

Advances in the use of ionic groups in the gel matrix of polyacrylamide gels have been reported from Polysciences, Inc. (NO1-CP-23245). It has been found that better gel properties are achieved by the incorporation of a zwitterionic unit, as opposed to a single negatively-charged molecule. The improvement is principally related to much greater reproducibility in making

such gels. Use of a short segment of such charged gel as a "spacer" preceding the main portion of the gel has made improved resolution of tRNAs possible. Identification of specific tRNAs is in progress.

Chemical carcinogens as agents that alter the translational components of cells resulting in changes in mRNA expression and the mechanism of action of chemical carcinogens on viral expression and cell transformation are being explored at the Weizmann Institute of Science (NO1-CP-33220). Techniques have been developed which may identify these mechanisms. They are: (1) a simple procedure for demonstrating the presence or absence of Y base of $tRNA^{Phe}$ and (2) a procedure for isolating viral mRNA by hybridization to immobilized viral DNA. The latter technique has the potentiality of being used for screening for the presence of viral sequences in tumor cells, including chemically-transformed cells. In addition, these studies have (1) shown a correlation between the absence of Y base of $tRNA^{Phe}$ and cancer cells which may be used as a screening assay for human cancer and (2) isolated SV40 mRNAs coding for three proteins, one of which codes for the major capsid protein and a second for T antigen.

Surface Membranes

The properties of the cell membranes which control the passage of materials in and out of the cell and which regulate their intracellular distribution may play a significant role in the development of malignancy. The Segment has undertaken collaborative studies to obtain more information in this area.

Investigation of special factors of transport systems in both the plasma membrane and the mitochondrial membrane of tumor cells is under way at The Johns Hopkins University Medical School (NO1-CP-45610). Calcium transport across both the plasma membrane and mitochondrial membrane has been examined in a number of tumor types. Tumor cells show a very small calcium influx; mitochondria from these cells showed very high transport and retention of calcium. The cytosol calcium concentration in tumor cells is more tightly regulated than in normal cells.

Glucose transport across membranes in growing and density-inhibited cells is being investigated at the Hebrew University (NO1-CP-43307). The results show important differences in the kinetic parameters. The results are interpreted to show two-compartment kinetics. Such studies will form a base line for subsequent studies of malignant cells.

The initial studies at the University of Rochester (NO1-CP-45611) have been directed at the synthesis of phenylalanine photoprobes which can be recognized by the transport system which handles phenylalanine. These may subsequently be activated to bind covalently to macromolecules for which the probe shows preferential binding, thus identifying the components of the transport system. Many of the steric constraints have been recognized and analogs synthesized which carry photosensitive groups. It has been possible to inactivate irreversibly several transport systems with one probe.

Human prolactin has been isolated and purified from frozen human pituitary glands, from acetone dried pituitary powder from human pituitaries and from amniotic fluid at the University of Manitoba (NO1-CP-43250). The material has been characterized by acrylamide gel electrophoresis and by isoelectric focusing. The material purified from amniotic fluid appears to be very similar if not identical with human pituitary prolactin with respect to molecular size (gel filtration), electrophoretic mobility (acrylamide gels), immunological criteria (parallel inhibition in radioimmunoassay) and amino acid composition (the first 25 residues are identical). The action of prolactin on mammary membranes seems to be important in the development of breast carcinoma. The Segment has arranged for this laboratory to provide human prolactin to facilitate such studies.

Metabolism and Carcinogenic Action of Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons are a major environmental hazard because so many of them are carcinogenic. To explore the host conditions that result in activation of these compounds, studies on the nature of the enzymes responsible for the conversion, the kinds of derivatives which are formed, and their mode of attachment to DNA are supported.

Synthesis of derivatives. To facilitate the identification of natural metabolites a number of derivatives of known structure are being prepared to serve as reference compounds. The major synthesis effort for polycyclic hydrocarbon derivatives is with Midwest Research Institute (NO1-CP-33387) which has supplied specimens of 33 compounds for use at NCI and some 50 other laboratories. Stability is monitored and physical and chemical parameters for each derivative are determined to facilitate analysis by laboratories using the material. It is expected that this contract will serve as a resource for the increasing needs of laboratories studying chemical carcinogenesis.

Several ancillary contracts designed to investigate specific chemical problems and develop new and innovative chemistry have supported the major contracts. Studies at the University of Chicago (NO1-CP-33385) have produced new benzo[a]pyrene phenols, hydroquinones, and work is in progress on novel NMR interpretation of phenol spectra to be used as an analytical tool.

Omni Research, Inc. (NO1-CP-33386) has been developing less destructive methods for tritiation of hydroxylated polycyclic aromatic hydrocarbons in order to maintain their structural integrity. These results have been transmitted to Midwest Research Institute (NO1-CP-33387) to aid in the preparation of 3-OH-BP-^{3}H.

Development of mutant bacterial strains which will transform carcinogenic polycyclic aromatic hydrocarbons into hydroxylated derivatives is being carried out at the University of Texas (at Austin) (NO1-CP-33384). This method has been successful for aromatic hydrocarbons of two and three rings. At present efforts are being made to develop a strain of bacteria that will survive in the presence of high concentrations of benzó[a]pyrene.

Metabolic studies. A variety of carcinogens and noncarcinogens have been studied at the Weizmann Institute of Science (NO1-CP-02217) in the system of

cell-mediated mutagenesis developed last year. Results with this animal system indicate that the degree of mutagenicity is related to the degree of carcinogenicity. This system may prove useful in the evaluation of environmentally hazardous chemicals. Studies on banded chromosomes have continued and appear to indicate that specific chromosomes are either added or deleted in malignant transformation and reversion. In addition, it has been found in some cell lines that polycyclic hydrocarbon metabolism is regulated by levels of dibutyrylcyclic AMP.

Preliminary experiments on the analysis of the mixed-function oxydases by electron paramagnetic resonance (Albert Einstein College of Medicine, NO1-CP-55606) are very promising. These experiments suggest that it may be possible to quantitate various types of oxygenase in crude biological materials involved in carcinogen metabolism. This would constitute an important development for the characterization of these enzymes and the study of their regulation.

Studies at the University of California at Berkeley [ERDA-NCI Interagency Agreement (YO1-CP-10058)] emphasized benzo[a]pyrene and indicate that although benzo[a]pyrene intercalates into poly G in aqueous solution, covalent attachment requires the action of aryl hydrocarbon hydroxylase. Fluorescence spectra are used to study the reaction. Various tritiated derivatives of BP have been prepared. These derivatives will be helpful in studying the chemical linkage involved in binding to DNA. This laboratory is also studying AHH in lines of epithelial cells and has found several lines which differ with respect to inducibility and cytotoxicity by BP.

Studies at the Institute of Cancer Research/Chester Beatty Research Institute (NO1-CP-33367) have continued to define the natural sequence of reactions leading to binding of benzo[a]pyrene to DNA. The DNA products produced by an in vitro reaction with various derivatives are compared with those formed by reactions between the parent hydrocarbon and mouse embryo cells. The 7,8-dihydrodiol gave the most extensive binding to DNA. The results are consistent with a diolepoxide intermediate being responsible for in vivo binding. This active compound was a minor product of the total metabolism.

Work at Ohio State University (NO1-CP-43248) is continuing the preparation of 7,12-dimethyl benz[a]anthracene attached to a polymer by a non-biodegradable link. This material will be used to investigate whether tumors can arise by reactions which take place outside the cell.

Studies on aryl hydrocarbon hydroxylase in humans. These studies were undertaken to evaluate reports that inducibility of aryl hydrocarbon hydroxylase in human lymphocytes was a heritable trait transmitted by Mendelian genetics and that lung cancer patients had genetically determined high levels prior to disease. The initial phase of the studies was concerned with the methodology and reproducibility of lymphocyte culture and enzyme assay (University of Texas [M. D. Anderson Hospital], NO1-CP-55604; Microbiological Associates, NO1-CP-55605; Roswell Park Memorial Institute, NO1-CP-55629). A blind study carried out by the M. D. Anderson group showed such poor reproducibility between samples obtained from a test person at a given day and between samples obtained at different times that an evaluation of genetic

control could not be made. Similar reproducibility problems were encountered by the groups at Roswell Park Memorial Institute and Microbiological Associates. A series of methodological questions have to be resolved before the human genetics of the enzyme system or its relationship to the occurrence of lung cancer can be studied in lymphocytes.

Somatic Cell Genetics

Because of the high probability that malignancy involves changes in the genome, the Segment is continuing its efforts to study the genetics of mammalian somatic cells. Two general types of activity have been supported: (1) isolation and characterization of mutants of somatic cells, and (2) mutagenesis of somatic cells in relation to carcinogenesis. The Ontario Cancer Institute (NO1-CP-43331) and New York University (NO1-CP-33279) have shown that both nonconditional and temperature-conditional mutants can be obtained from cells in culture. The specific defect in two mutants (temperature-sensitive enzymes, leucyl-tRNA synthetase and asparaginyl tRNA synthetase) was identified. A third mutant was defective in processing of ribosomal RNA. A number of antimetabolite-resistant mutants have been isolated and are being characterized to localize the defects. When a larger number of mutants have been characterized for specific defects, studies on mutagenesis directed toward analyses of chemical carcinogens can be made.

The most common mutations studied in investigating correlations between chemical carcinogenesis and mutagenesis are those which give rise to defects in purine metabolism (azaguanine resistance). Interpretations of such data are somewhat tentative because of the complexity of the purine metabolic pathway. Definitive resolution of these pathways by selection and characterization of specific mutants is in progress at the University of California at San Francisco (NO1-CP-43239).

The consequences of mutation in the genome can be detected by study of gene products much more decisively in haploid cells than in diploid cells where an unaffected allele may obscure the results. Investigations at Yale University (NO1-CP-55673) are attempting to obtain fully haploid cells, and have been successful in acquiring cell lines which have lost sufficient genetic material to become haploid in certain genetic regions.

The application of genetic techniques to chemical carcinogenesis studies in cell culture (University of Illinois [NO1-CP-23303]) has led to a method for testing the mutagenic action of certain chemical carcinogens. Chemicals which exert their mutagenic action by base substitution reactions in DNA should produce mutants, a high proportion of which are temperature-dependent cellular transformations, while chemicals causing base additions or deletions should produce mostly nonconditional transformations. Both nonconditional and temperature-dependent transformations have been demonstrated.

Study Involving Genetics of Haptoglobin in Patients with Cancer of the Pancreas

Final reports from contracts, which terminated last fiscal year, with the University of Texas (NO1-CP-]2268) and with the Home for the Jewish Aged, Philadelphia (NO1-CP-33274), on the distribution of Hp genes in patients with

pancreatic cancer were in general agreement that, although the frequency of the Hp^1 phenotype was higher than in control groups, the differences were not great enough to be statistically significant. The biggest differences were found between female patients and controls. There was no difference between males and females in the control group. Cancer of the pancreas is a sex-discriminant disease, being more common in males. Therefore, the finding of a sex difference in haptoglobin gene distribution is provocative, but ambiguous.

At the University of Texas a large number of subjects with various diseases were studied, and the patients with pancreatic cancer had the highest frequency of gene 1. The excess was in heterozygotes. At the Home for the Jewish Aged in Philadelphia, the excess in female patients was also in the heterozygotes. Like other findings in this study, it is unclear what significance these finds have.

It was clear, however, that the association between haptoglobin-type and pancreatic cancer is too tenuous to permit practical utilization in identifying persons at increased risk, and the contracts have been closed.

CONTRACT NARRATIVES

CHEMISTRY AND MOLECULAR CARCINOGENESIS SEGMENT

July 1, 1974 through June 30, 1975

ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1-CP-45606)

Title: Studies on Microsomal Enzyme Studies Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans

Contractor's Project Director: Dr. Jack Peisach

Project Officers (NCI): Dr. Harry V. Gelboin
Dr. F. J. Wiebel

Objective: To develop a magnetic assay for that cyrochrome P-450 in mammalian liver which is the enzyme involved in aryl hydrocarbon hydroxylase (AHH) activity. To achieve this goal, rats were induced for this particular enzymic activity using two different inducers: 3-methyl cholanthrene (3-mc) and Sudan III, an azo-dye.

Major Findings: In both instances, AHH activity reached the same maximum value over controls in some isolated preparations, but the total cytochrome P-450, as measured optically was different. A comparison of electron paramagnetic resonance (EPR) spectra from liver microsomes, from controls, 3-mc- or Sudan III-induced animals showed a heterogeneity of cytochromes P-450 in each preparation in which the levels of the individual cytochromes P-450 are each different. It was found that the levels of an individual cytochrome P-450 which is found in each of these preparations is directly related to AHH activity. A preparation of soluble cytochrome P-450 from rats induced with 3-mc which was found to be homogeneous in hemoprotein and to have AHH activity, exhibits the EPR of a single cytochrome P-450. The g values of this cytochrome are the same as the g values of the AHH in the microsomal preparation. It has thus been demonstrated that a single cytochrome P-450 in liver microsomes is primarily involved with AHH activity.

Significance to Biomedical Research and the Program of the Institute: These results facilitate the development of a non-degradative assay for AHH using a biophysical probe.

Proposed Course: These studies will be continued using soluble preparations from rabbit liver in which the AHH activity per concentration of cytochrome P-450 is greatly diminished from that which is observed for rat. In addition, preliminary studies show that soluble cytochrome P-450 prepared from 3-mc-induced rabbits contains a large amount of high spin material even in the absence of substrate which is not found in rat preparations. A future aim of this study is to ascertain the role of high spin cytochrome P-450 in the hydroxylase activity. Finally, efforts will be made to develop a magnetic assay for that cytochrome P-450 in mammalian cells responsible for AHH activity.

Date Contract Initiated: August 6, 1974

Current Annual Level: $60,000

CALIFORNIA, UNIVERSITY OF (at San Francisco) (NO1-CP-43239)

Title: Selection and Propagation of Somatic Cells Having Specific Physiological Mutations

Contractor's Project Director: Dr. David W. Martin, Jr.

Project Officer (NCI): Dr. John P. Bader

Objectives: To answer two very important technical questions about the isolation of conditional mutants and then to provide biochemically characterized chemically-induced conditional and nonconditional mutants in which the processes responsible for the regulation of purine biosynthesis and thus cell growth and division have been altered.

Major Findings: Somatic cell genetics are utilized to study the regulation of purine synthesis in a line of cultured rat hepatoma cells. From a population of chemically mutagenized cells a clone ("1020/12") has been selected for resistance to 6-mercaptopurine. The catalytic activity of hypoxanthine-guanine phosphoribosyltransferase (HPRT) in 1020/12 cells is 40 percent that of wild-type cells. Cells of clone 1020/12 overproduce purines de novo and possess three- to four-fold elevated concentrations of phosphoribosyl-pyrophosphate (PRPP) synthetase, the rate-limiting enzyme for purine synthesis. The PRPP synthetase molecules are apparently of the wild-type, but immunologic studies have demonstrated that the cells possess a mutation in the structural gene for HPRT.

It is proposed that the structural gene for HPRT is bifunctional: it codes for the catalytic function of HPRT activity and, in addition, regulates the intracellular concentration of PRPP synthetase molecules and, thus, the rate of purine synthesis.

As a test of this hypothesis, a series of mutant hepatoma cells have been isolated by independent means, each possessing a mutation in the gene for HPRT. Of the ten independent clones, nine exhibit significantly increased concentrations of PRPP synthetase. The degrees of increased PRPP synthetase activities do not correlate in any simple manner with the degrees of deficiencies of HPRT catalytic activities. Studies are underway to determine the molecular mechanisms responsible for the regulation of cellular concentrations of PRPP synthetase and thus rates of cellular proliferation.

Significance to Biomedical Research and the Program of the Institute: Because of the obvious importance of both the de novo purine biosynthetic pathway and the purine salvage pathways in control of cell growth and division, the isolation and characterization of mutants in these pathways will play a very significant role in the understanding of both normal and malignant growth regulatory processes, the mechanisms of carcinogenesis and the pharmacology of cancer therapy.

Proposed Course: The proposal has been divided into four phases. Phase I, the determination of the proper approach for the isolation of specific thermosensitive nonlethal mutants is well under way and data are currently

being collected. Phase II, the design of effective killing system to eliminate cells which are capable of dividing under conditions where the desired mutants cannot, has been completed by demonstrating that exposure to high concentrations of cytosine arabinoside and fluorodeoxyuridine for short periods of time is effective. Phase III, the completion of the biochemical characterization of those potential regulatory mutants already isolated and the selection, isolation and characterization of additional regulatory mutants is well under way. Phase IV, in which the approaches and techniques of phases I, II and III will be utilized to select, isolate and characterize thermosensitive regulatory mutants, will commence within the next twelve months.

Date Contract Initiated: March 25, 1974

Current Annual Level: $106,628

CHICAGO, UNIVERSITY OF (NO1-CP-33385)

Title: Synthesis of Derivatives of Carcinogenic Polycyclic Hydrocarbons

Contractor's Project Director: Dr. Ronald G. Harvey

Project Officer (NCI): Dr. James K. Selkirk

Objectives: To synthesize polycyclic hydrocarbon derivatives and metabolites to be available to the scientific community for in vivo and in vitro studies in carcinogenesis. These compounds are of special value in studying enzyme pathways in chemical carcinogenesis.

Major Findings: (1) Preparative scale syntheses have been accomplished on the following compounds: DMBA 5,6-oxide, 7, 12-bis(hydroxymethyl)DMBA, BP-3, 6-quinone, BP-1, 6-quinone, BP-4,5-quinone, BA-5,6-quinone,1,6-diacetoxy-BP, 3, 6-diacetoxy-BP, DMBA-5, 6-quinone. Additional compounds synthesized successfully on smaller scale and in preparation on larger scale include: 4-HO-BP, 5-HO-BP, 1-HO-BP, 7-HO-BP, 5-HO-BA, 6-HO-BA, and 7, 8-dihydroxy-7, 8-dihydro-BP. (2) Separation of isomeric K-region phenols has been accomplished in the case of 5- and 6-HO-BA, but found not to be a practical route in general due to the facility of decomposition of the phenols, as well as their acetates and methyl ethers, on chromatography. The use of other protecting groups is being explored, while the pure K-phenols of BP and DMBA (keto form) have been prepared by individual total synthesis. The non-K-phenols (except 6-HO-BP) such as 1-, 3-, and 7-HO-BP, were found to be relatively stable. (3) Synthesis of 1-HO-BP from BP by a more convenient route and in much higher yield than recently reported by Jerina has been achieved. An unusual side product was identified as a BP-lead derivative. This type of compound which was used to prepare Me_2-BP may prove to be a useful synthetic intermediate. (4) Synthesis of 7-HO-BP was accomplished in high yield by a method superior to that reported. (5) Synthesis of 12-HO-BP by adaptation of the procedure for synthesis of 4-HO-BP requires only the last cyclization step to be complete. (6) Synthesis of the hydroquinones of polycyclic hydrocarbons has been accomplished by what is the first general method for this purpose, reduction of the quinones with $NaBH_4$ in

dimethylacetamide. A large series of hydroquinone acetates, including seven derivatives of BA and BP have been made by this method. (7) The 7,8-diol of BP has been synthesized as a pure crystalline material in high yield from 9,10-dihydro-BP. Synthesis of the related 9, 10-oxide is in progress. (8) Other syntheses include an efficient one-step OsO_4-catalyzed oxidation of DMBA with sodium periodate to furnish the dialdehyde derived from oxidation of the K-region. Other compounds which are probable carcinogens or carcinogen metabolites which have been synthesized include: 5-methylchrysene, 5-bromomethylchrysene, 12-HO-CH_2-BA, 12-bromomethyl-BA, 12-formyl-BA, 7-formyl-12-methyl-BA, 6-formyl-BP, 6-methyl-BP, 6-HO-CH_2-BP, 6-bromomethyl-BP. (9) Synthesis of ^{3}H-BP-4, 5-oxide via the corresponding cisdiol, quinone, and transdiol was carried through successfully. (10) High resolution nmr spectroscopy is being utilized to aid in structural assignment of the isomeric products and elucidate details of stereochemistry and molecular structure. It is planned to develop this powerful tool to aid directly in the analysis of the structures of carcinogen metabolites.

Significance to Biomedical Research and the Program of the Institute: The known or suspected carcinogens and metabolites of carcinogens synthesized in this project are of urgent need in carcinogenesis research. It would be virtually impossible for a laboratory lacking specific synthetic experience to synthesize any one of these compounds for a specific experiment. The combined contract of which this project is part will put the National Cancer Institute in the position of providing compounds for pertinent experiments in chemical carcinogenesis which could not be carried out otherwise.

Proposed Course: Compounds for which syntheses have been already devised will be prepared in the required scale, and exploratory experiments to develop convenient syntheses of other compounds sought will continue. Particular emphasis will be given to completing the preparation of all the remaining benzo[a]pyrene phenol isomers and developing satisfactory syntheses of the non-K-region arene oxides, etc., particularly the unknown A-ring derivatives.

Date Contract Initiated: June 20, 1973

Current Annual Level: $81,251

CONNECTICUT, UNIVERSITY OF (at Storrs) (NO1-CP-23268)

Title: Isolation and Characterization of Non-Histone Proteins Binding DNA

Contractor's Project Director: Dr. Jay S. Roth

Project Officer (NCI): Dr. C. Wesley Dingman

Objectives: To develop and explore new techniques for isolating from mammalian cells non-histone proteins which show a high affinity for DNA, particularly homologous DNA since such proteins have the *a priori* possibility of being important in the regulation of genetic transcription.

Major Findings: Various methods for making DNA-cellulose are being explored. Successful preparations will then be used to chromatograph protein fractions and separate out those showing affinity for homologous DNA. Simultaneously, four techniques for the initial bulk isolation of non-histone nuclear proteins have been explored to date. Two of these techniques show some promise and the proteins obtained from them (using rat liver as the source) are currently being further characterized as to their variety and affinity for DNA.

Significance to Biomedical Research and the Program of the Institute: The regulation of both DNA replication and DNA transcription involves proteins which show a specific affinity for homologous DNA. In many cases this affinity for DNA probably involves specific nucleotide sequences in the DNA. The future understanding of the control of DNA replication and transcription, both in normal and malignant cells, depend upon the ability to isolate and characterize those proteins involved in these processes. This project, if successful, should be of great help to those investigators interested in exploring the possibility that some aspects of cellular behavior, including malignancy, can be explained by alterations in the kind or amount of proteins which function by virtue of their specific binding to DNA.

Proposed Course: Different subcellular fractions will be tried as sources for these proteins and different chromatographic techniques will be applied in attempts to find the best possible means for isolating and fractionating the largest possible set of cell-specific, DNA-binding, non-histone proteins.

Date Contract Initiated: June 29, 1972

Current Annual Level: $34,646

ERDA-NCI INTERAGENCY AGREEMENT (University of California at Berkeley) (Y01-CP-10058)

Title: Molecular Processes Involved in the Carcinogenic Action of Polycyclic Aromatic Hydrocarbons

Contractor's Project Director: Dr. Melvin Calvin

Project Officer (NCI): Dr. Andrew C. Peacock

Objectives: To define the chemically reactive positions of polycyclic hydrocarbons and to determine the interaction of the reactive intermediates with cell macromolecules and the nature of interaction with oncogenic viruses.

Major Findings: During 1974 investigations of the complexing of polycyclic aromatic hydrocarbons (PAH'es) with synthetic and natural polynucleotides continued. A series of experiments were completed on the interaction of pyrene, benzo[a]pyrene (BP) and benzo[e]pyrene with different nucleotide systems. The method of analysis has been primarily spectroscopic. The question that is being asked is whether an alteration in the excitation and/or emission spectra of these PAH'es can be detected when they are examined in the presence of various polynucleotide systems. These shifts in spectra would

indicate that a physical interaction between the PAH and the polynucleotide system was occurring. The only significant amount of interaction that can be seen using this technique is between BP or benzo[e]pyrene and poly G. No interaction was seen between pyrene and any of the polynucleotide systems.

The next step is to look at the binding of BP to polynucleotide systems by following radioactively labeled BP. The approach has been to study the binding of BP to synthetic polynucleotides. The rationale for using this system is that synthetic polynucleotides are readily available, well defined in composition, and can be obtained in reasonably large quantities. It has been shown by other workers that the efficiency of covalent linkage of PAH'es to cellular polynucleotides is very low, making determination of the structure of the carcinogen-nucleotide complex very difficult. Synthetic polynucleotides offer the possibility of using highly repetitive reaction sites to obtain enough carcinogen covalently bound to do chemical analysis.

Experiments thus far have demonstrated that BP intercalates into poly G in an aqueous solution. Covalent binding of BP to poly G requires the action of aryl hydrocarbon hydroxylase. With this system enough BP can be bound to poly G to obtain a fluorescence excitation and emission spectrum.

At the same time, techniques have been developed to study the interaction of PAH'es with biological macromolecules. Any investigation of how PAH'es interact with biological systems is difficult because of the complexity of the interactions and the number of components involved. Attempts to overcome some of these difficulties include studying the interaction of PAH'es with viruses. SV40 and polyoma are well-characterized viruses composed mainly of DNA and protein. Both the DNA and the protein components have been isolated and studied. The experiments to be undertaken require growth of the virus in cells that can metabolize the PAH'es. Unfortunately, the monkey cells which support the growth of SV40 do not metabolize the BP. Mouse epithelial cells do actively metabolize the carcinogen. These cells will be described in more detail later. The mouse epithelial cells do not support the growth of SV40, but polyoma virus will multiply in these cells. Polyoma virus grown on these epithelial cells is being purified. This system will be used to try to answer the following questions: Do PAH'es effect the production of either RNA- or DNA-type viruses? What are the effects of PAH'es on the number of DNA breaks as measured by the ratio of supercoiled DNA to relaxed circular and linear DNA? Can a chemical linkage be demonstrated between carcinogen and DNA and/or proteins of polyoma? And, finally, what is the effect of inhibitors of PAH'es metabolism on the interaction of PAH'es with viruses?

The action of BP on different cell lines of both epithelial and fibroblastic origin is under investigation. There have been a number of reports that the cytotoxicity of BP and other PAH'es to different cell lines is a function of the ability of the cells to metabolize the hydrocarbon to hydroxylated derivatives. The relationship between cytotoxicity of hydrocarbons and their ability to transform cells is not clear. It is clear, however, that aryl hydrocarbon hydroxylase (AHH) is involved with transformation. Results indicate that different cell lines respond differently to BP in their growth medium and that all the epithelial cell lines tested were more sensitive to the carcinogen than the fibroblastic cell lines.

In studying the metabolism of PAH'es in various cell lines, it has been found that cells showing cytotoxicity to hydrocarbons and induction of the AHH also are capable of converting the hydrocarbons into fluorescent derivatives which can be detected in the cells and in the medium surrounding the cells. Following these observations, attempts have been made to develop a method of assaying hydrocarbon metabolism in single cells by measuring the amount of fluorescence of the metabolic products in each cell. The technique is a modification of flow microfluorimetry and uses a laser tuned to the UV to excite the derivatives which, at alkaline pH's, fluorescence at wavelengths well separated from that of the parent compound. This green fluorescence has been detected both by fluorescent microscopy and flow microfluorimetry. There are some difficulties with cell handling at alkaline pH'es in that there is considerable clumping and cell lysis leading to efforts to develop optimal conditions for the fluorescence to appear and the cells to remain intact.

Significance of Biomedical Research and the Program of the Institute: The widespread distribution of polycyclic hydrocarbons as a result of the combustion of fossil fuel, both in moving and in stationary power plants, as well as in the combustion of agricultural wastes, makes them important environmental carcinogens. Their presence as a principal component of tobacco smoke whose carcinogenicity has been clearly demonstrated epidemiologically makes it even more critical to know their mode of action--the role of enzymes in the activation as well as in the binding of the carcinogen, the cellular components and the components of oncogenic viruses. A first step has already been taken in helping to understand how these hydrocarbons may become active.

Proposed Course: To investigate the metabolic activation of polycyclic aromatic hydrocarbons and the subsequent transformation of mammalian cells. The research is interdisciplinary in nature and, consequently, approaches a similar problem from a number of different directions.

Date Contract Initiated: April 12, 1971

Current Annual Level: $80,000

ERDA-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) (Y01-CP-50200)

Title: NCI-ERDA Carcinogenesis Program

Contractor's Project Director: Dr. Francis T. Kenney

Project Officer (NCI): Dr. Allen Heim

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

HEALTH RESEARCH INC./ROSWELL PARK MEMORIAL INSTITUTE (NO1-CP-55629)

Title: Aryl Hydrocarbon Hydroxylase in Human Lymphocytes and the Relationship to Chemical Carcinogenesis

Contractor's Project Director: Dr. Kenneth Paigen

Project Officers (NCI): Dr. Friedrich Wiebel
Dr. Ann Kaplan

Objective: The objective of this contract is to confirm a report in the New Eng. J. Med. 289: 934, 1973 that aryl hydrocarbon hydroxylase (AHH) inducibility is a common polymorphism and that persons with intermediate or high inducibility have a higher risk of lung cancer.

Major Findings: A reproducible assay has been developed that is about 20 times more sensitive by changing both the lymphocyte culture and enzyme assay conditions, yielding results in a range of 1.0 to 4.6 for AHH inducibility in the normal population using methyl cholanthrene as inducer and benzopyrene as substrate. About 10 percent of individuals are above 4.0. Most individuals give a coefficient of variation of 0.1 for repeat determinations, but 10 percent of individuals are not reproducible. Lung cancer patients are being studied to determine whether their AHH inducibility is different from normals.

Significance to Biomedical Research and the Program of the Institute: Polycyclic hydrocarbons, an important class of environmental carcinogens, are found in cigarette smoke, polluted air, and food. These carcinogens are metabolized primarily by the inducible enzyme aryl hydrocarbon hydroxylase, found in most mammalian tissues. If people vary in their ability to induce this enzyme, if this variation is genetically determined, and if lung cancer occurs primarily in people with intermediate or high inducibility, then several pathways are opened for cancer control. High-risk individuals could be identified and advised to give up smoking, to avoid high risk occupations, and to seek regular medical checkups.

Proposed Course: To continue studies of lung cancer patients and to initiate family studies of aryl hydrocarbon hydroxylase genetics.

Date Contract Initiated: August 12, 1974

Current Annual Level: $62,000

HEBREW UNIVERSITY (NO1-CP-43307)

Title: Mammalian Cell Transport Systems: (3) Influence of Intercellular Factors on Plasma Membrane

Contractor's Project Director: Dr. Wilfred D. Stein

Project Officers (NCI): Dr. Tsuyoshi Kakefuda
Dr. Ann Kaplan

Objectives: (1) Obtain reliable data and trustworthy kinetic parameters for the uptake of cell nutrients and non-metabolisable analogues of these nutrients for a number of different normal cell lines and from the malignant cells derived from these, following viral or chemically-induced transformation. (2) Study the time course of emergence of the new transport kinetics following cell transformation to the malignant state. (3) Evaluate the influence of cell density on transport properties to determine the contribution of cell-cell interactions.

Major Findings: (1) The sugar transport system of BHK cells grown in culture undergoes a dramatic change as the cells reach a confluent density. The rapid, saturable system found in growing cells is replaced by a slow non-saturable system, the change occurring in only certain cells at any time. (2) The uridine transport system of 3T3 fibroblasts is activated following stimulation of quiescent cells by serum. Activated and non-activated cells display the same half-saturation concentration for uridine and the same temperature dependence for the maximum velocity of transport. Apparently a change only in the number and not in the nature of the uridine transporters is found following activation.

Significance to Biomedical Research and the Program of the Institute: The events studied are closely associated with the early events of the cessation of cell growth following confluence and the reactivation of growth on stimulation. They are hence directly associated with the question of growth control, the key problem of cancer. An understanding of the processes concerned in the switching on and off of membrane transport must be basic to the understanding of growth control and its malfunction.

Proposed Course: To study in detail the factors involved in switching off sugar transport in BHK cells and to study the detailed events in the switching on of transport in 3T3 cells. Transport events will be monitored, and labeled membrane proteins separated and analysed.

Date Contract Initiated: June 25, 1974

Current Annual Level: $65,087

ILLINOIS, UNIVERSITY OF (NO1-CP-23303)

Title: Temperature Sensitive Mutants in In Vitro Carcinogenesis

Contractor's Project Director: Dr. Giampiero di Mayorca

Project Officer (NCI): Dr. John P. Bader

Objectives: Expose BHK 21 hamster cells to chemical carcinogens. Analyze transformation by microscopic observation of morphological change and altered ability to grow under normally restrictive conditions, such as sensitivity to temperature. Manipulate clones of transformed cells for ability of plating reasons for physiological change. Test transformed cells for ability of plating in semi-solid medium and tumorigenesis in the hamster.

Major Findings: Substantial evidence obtained suggests the mechanism of cell transformation by chemical carcinogenesis is a mutagenic one. Inhancement of transformation frequency by chemical carcinogens in cells infected with leukemia viruses might be the consequence of an impairment of the DNA repair mechanism of the cell.

Significance to Biomedical Research and the Program of the Institute: An exploitable system for the determination and analysis of chemical carcinogens is essential to progress in understanding carcinogenesis with the ultimate view of preventing and/or curing tumors induced by chemicals.

Proposed Course: Biochemical characterization of the conditionally transformed cells will be carried out particularly in regard to the cell membrane proteins in the attempt to define changes brought about by the conditional state. A parallel investment will be carried out by immunological detection of the cell surface antigens.

Date Contract Initiated: June 20, 1972

Current Annual Level: $156,635

INSTITUTE OF CANCER RESEARCH (NO1-CP-33367)

Title: Nature of the Polycyclic Hydrocarbon--Nucleic Acid Compound in Hydrocarbon Carcinogenesis

Contractor's Project Director: Dr. Peter Brookes

Project Officer (NCI): Dr. Harry V. Gelboin

Objectives: To understand the chemistry of the reaction between carcinogenic hydrocarbons and the DNA of mammalian cells which results when these compounds are metabolised by the cells. Furthermore since it now seems probable that more than one reaction product is present in the DNA, the significance of each reaction will be considered in relation to mutagenesis and transformation.

Major Findings: Pre-labelling of the DNA of mouse embryo cells with the appropriate tritium labelled purine or pyrimidine base precursors followed by treatment of the cells with either 7-methyl-benz[a]anthracene (BA) or benzo-[a]pyrene (BP) has established the major in vivo hydrocarbon products to be purine derivatives.

3-Methylcholanthrene-induced rat liver microsomes were used to catalyse the binding of tritium of carbon-14 labelled BP, or its 4,5- 7,8-, or 9,10-dihydrodiol derivatives to added DNA. Enzymic degradation of the resulting DNA samples to deoxyribonucleoside fragments followed by chromatography on an LH 20 Sephadex column enabled the various hydrocarbon-deoxyribonucleoside products to be compared with those obtained from DNA having BP bound following treatment of mouse embryo cells, and from DNA reacted with BP-4, 5-oxide (K-region epoxide). The aryl hydrocarbon hydroxylase activity of the microsome

preparations was determined and related to the extent of hydrocarbon binding. The level of DNA binding obtained with BP-7, 8 dihydrodiol was much greater than that obtained with BP or the other diols used, in confirmation of the report in the J. Med. Chem., 16: 502, 1973. The use of epoxide hydrase inhibitors implicated this enzyme in the series of reactions leading to the in vivo hydrocarbon-DNA product (i.v.p.). The i.v.p. was shown to be only a minor product of microsome catalysed BP-DNA binding but was the only product when the 7,8-dihydrodiol was used. The results are consistent with a diolepoxide intermediate being responsible for the in vivo binding as suggested in FEBS Letters: 47: 34, 1974 for BA.

The hydrocarbon metabolizing activity of purified rat liver nuclei was compared with that of liver microsomes. After pre-treatment of the rats with 3-methylcholanthrene, liver nuclei and microsome hydroxylation gave essentially the same pattern of BP metabolites, and the same DNA-bound products were obtained with either added DNA (in the case of microsomes) or endogenous DNA (in the case of nuclei). Phenobarbitone pre-treatment did not significantly increase the overall hydroxylase activity of nuclei but led to an increase in the relative proportion of the 4,5-diol metabolite.

Preliminary results indicate that the in vivo binding of BP to the DNA of V79 Chinese hamster cells, mediated via metabolism by BHK cells, is an efficient mutational event, being quantitatively more effective than mutation by the K-region epoxide derivative of BP.

Significance to Biomedical Research and the Program of the Institute: A complete understanding of the chemistry of the in vivo reaction between carcinogenic hydrocarbons and DNA will indicate the nature of the reactive species mediating this reaction and hence suggest ways in which the production of this moiety could be inhibited. The nature of the genetic alterations consequent on hydrocarbon-DNA reaction, as revealed by mutation studies, should advance understanding of the molecular basis of neoplasia and further advance knowledge of the types of compound which might be expected to elicit this response.

Proposed Course: In the case of BP the available evidence strongly implicates the 7,8-diol-9, 10-epoxide as being the metabolite leading to the in vivo DNA binding. The chemistry of the reaction of this compound with DNA will be investigated to firmly establish its role in the in vivo binding and hopefully to indicate why this particular DNA reaction is of such biological relevance.

Date Contract Initiated: June 25, 1973

Current Annual Level: $65,000

JOHNS HOPKINS UNIVERSITY (NO1-CP-33276)

Title: Regulation of the Heme Moiety of P-450 in Relationship to Carcinogen Metabolizing Activity

Contractor's Project Directors: Dr. Ephraim Y. Levin
Dr. Peter R. Adams

Project Officer (NCI): Dr. Harry V. Gelboin

Objectives: To determine the mechanism of regulation of the synthesis and degradation of the heme moiety of P-450, and to examine this regulation with respect to carcinogen metabolism.

Major Findings: In vitro studies of microsomal heme synthesis from labelled precursors have shown the presence of a component in liver cell sap which transfers mitochondrially-synthesized heme to microsomes. An assay method has been developed for this component, showing it to be active at low concentrations. Bovine serum albumin is relatively inactive at comparable protein concentrations.

Significance to Biomedical Research and the Program of the Institute: The heme transfer component may be instrumental in regulating the level of microsomal heme and, through this, the carcinogen-metabolizing activity of the cell. These aspects of cancer development should then be accessible to direct study.

Proposed Course: To determine the properties of the heme transfer component, and the effect of carcinogens on its role in microsomal heme synthesis.

Date Contract Initiated: April 30, 1971

Current Annual Level: $73,320

JOHNS HOPKINS UNIVERSITY (NO1-CP-45610)

Title: Mammalian Cell Transport Systems: (1) Influence of Membrane Mitochondrial Membrane Changes on Plasma Membrane Transport

Contractor's Project Director: Dr. Albert L. Lehninger

Project Officers (NCI): Dr. Tsuyoshi Kakefuda
Dr. Ann E. Kaplan

Objectives: To determine in what ways the specific transport systems of the mitochondrial membranes and plasma membranes of cancer cells are defective or show aberrations from normal behavior. Particular focus is on the role of the mitochondrial and extramitochondrial compartments in the regulation of energy-yielding metabolism and the acid-base balance of the cell in relation to the host organism. Moreover, it is hoped to ascertain to what extent these membrane changes are induced by oncogenic agents and to what extent they may be reversed.

Major Findings: Results show that the outer membrane of various ascites tumor cells contains a highly active transport system of broad specificity that carries lactate, the product of the breakdown of glucose by tumor cells, out

of the cell at a very high rate. It has long been known that tumor cells have an aberration in glucose metabolism that results in pouring out of a large amount of lactic acid. This lactate transport system, hitherto undescribed, has been studied in some detail. It transports only a few metabolites, in particular lactate and pyruvate, it is inhibited by certain analogs of lactate, namely α-cyanohydroxycinnamate and hydroxyphenyllactate, and transports either undissociated lactic acid (or lactate + H^+) down a concentration gradient.

The transport of Ca^{2+} across the plasma membrane has also been studied and the mitochondrial membranes of a number of tumors. These processes are relevant to tumor cell biology because the cytoplasmic Ca^{2+} ion concentration regulates many important intracellular activities. Tumor cells show very low rates of Ca^{2+} influx, but the mitochondria from the tumors show very high rates of transport and retention of Ca^{2+} and phosphate. From these and other points of evidence it appears that the Ca^{2+} concentration in the cytosol of tumor cells is more tightly regulated than in normal cells, possibly at a lower level, and may thus poise the activity of a variety of different Ca^{2+}-dependent processes in tumor cells at a different level than in normal cells.

Significance to Biomedical Research and the Program of the Institute: Cancer cells differ from normal cells in utilizing anywhere from 5 to 15 times as much glucose from the blood per unit weight per unit time as normal cells. They convert this excess glucose into lactic acid which appears in the blood. Little is known of the genetic or cellular changes that bring about this characteristic change, which in principle renders cancer cells parasitic on other normal cells in the body, thus robbing some normal tissues of sources of energy. Study of the membrane transport mechanisms responsible for carrying glucose into the cancer cell and lactic acid out during the process of carcinogenesis may help to unravel the nature of the metabolic regulatory mechanisms that undergo aberrations in cancer cells. Moreover, the inhibition of these transport processes may lead to some means of specific control of cancer cell growth or of the systemic effects of cancer on the host.

This project is aimed toward the solution of one of the problems facing the National Cancer Institute, namely, the definition of the factors and cellular processes that are altered in the process of carcinogenesis, whether spontaneous or induced by exogenous chemical agents. It may also have a long-range application to chemotherapeutic problems.

Proposed Course: To examine lactic acid transport more closely both in isolated tumor cells and in tumors growing in experimental animals, particularly to determine the effects of various inhibitory agents on lactic acid transport, with respect to cancer cell growth.

Investigations of Ca^{2+} transport by the mitochondria of tumor cells are very relevant to the regulation of the cell cycle of malignant cells, as well as the tendency of cancers to metastasize and lodge in normal tissues. Ca^{2+} concentration within cells controls the capacity of the cell to form junctions with other cells; there is reason to believe that this regulatory mechanism is defective in some types of tumors.

Date Contract Initiated: June 28, 1974

Current Annual Level: $99,807

MANITOBA, UNIVERSITY OF (NO1-CP-43250)

Title: Isolation, Purification and Characterization of Human Prolactin

Contractor's Project Director: Dr. Henry G. Friesen

Project Officers (NCI): Dr. Andrew C. Peacock
Dr. Marie Green

Objectives: The isolation, purification and characterization of human prolactin.

Major Findings: (1) Purification of pituitary prolactin--extraction and purification of pituitary prolactin has continued using methods which were developed by the contractor. The procedures have been applied to the purification of prolactin both from acetone-dried human pituitary powder and from frozen pituitary glands. The latter are a superior source for purification purposes because the yields of prolactin are considerably greater than those obtained from acetone-dried powder. The methods employed have proved to be reasonably satisfactory and have yielded a product with a potency of 25-30 IU/mg which is equal to that of sheep prolactin standards.

Amino acid sequence studies are being continued in collaboration with the Howard Florey Institute in Melbourne, Australia. To date, approximately one-half of the total amino acid sequence of human pituitary prolactin has been elucidated (4).

This year 2000 frozen pituitary glands have been obtained from the Medical Research Council of Canada for extraction of pituitary prolactin and 1500 from the National Pituitary Agency in Australia. In addition, a prolactin-rich side fraction has been extracted from 10,000 glands using the Raben procedure. This year four separate extraction procedures have been carried through on frozen glands, yielding approximately 25 mgs. of human prolactin from this source.

(2) Amniotic fluid prolactin--the aid of several colleagues has been enlisted to collect amniotic fluid for subsequent extraction and purification of prolactin. To date between 200 and 250 litres are on hand with an approximate concentration of 1 μg/ml, i.e. 200 mgs of human prolactin.

The procedures for purification of amniotic fluid prolactin include (a) standard precipitation procedures; (b) affinity chromatography; and (c) isoelectric focusing on a preparative scale. Being processed are 54 liters of amniotic fluid which contain 42 mgs. of prolactin.

The amino acid composition and partial amino acid sequence of one amniotic fluid prolactin preparation is being analyzed. The first 20 NH_2-terminal amino acid residues are identical with those of pituitary prolactin.

(3) Studies on prolactin receptor and application of radioreceptor assay--During the course of this contract, plasma membrane receptors for prolactin were identified in a number of tissues. In initial studies, results provided the identification and characterization of rabbit mammary cell receptors for prolactin. This resulted in a successful radioreceptor assay for prolactin and other lactogenic hormones. This assay proved to be very helpful in monitoring prolactin during the purification procedures. It proved to be a unique assay for a number of lactogens throughout pregnancy in several different species and very useful for structure-function studies of prolactin and other lactogens.

The particulate preparation containing the prolactin receptor has been solubilized and the receptor purified. The availability of a purified solubilized prolactin receptor should lead to more reproducible assay results. In addition, it should facilitate studies on structure-function relationships.

Significance to Biomedical Research and the Program of the Institute: The purification and characterization of human prolactin has significant implications to the overall problem of carcinogenesis. Prolactin has been implicated in the development of two of the most common types of cancer: mammary and prostatic carcinomas. The data linking prolactin to these two forms of cancer is extensive in experimental animals; in human subjects, the evidence is much less clear. One of the major reasons for the obscure relationship in man is based on the simple fact that human prolactin was not identified as a separate human pituitary hormone until three years ago. At the moment, a considerable amount of research on human prolactin is impossible because of the very limited amounts of hormone available. One of the major aims of this contract is to continue to explore methods which will improve purification procedures currently employed to increase the available supply of this hormone to allow more extensive research on human prolactin and its role in carcinogenesis to be carried out in man.

Proposed Course: The further purification and characterization of human prolactin.

Date Contract Initiated: June 30, 1972

Current Annual Level: $45,326

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP-55605)

Title: Studies of Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans

Contractor's Project Director: Dr. Richard E. Kouri

Project Officer (NCI): Dr. Friedrich J. Weibel

Objectives: To define the relationship between levels of the enzyme systems, aryl hydrocarbon hydroxylase (AHH), and cancer susceptibility in the human population. Emphasis will be on standardization of the AHH assay as it applies to the samples from Creighton University, participation in a study using a blind protocol, and determination of the relationship between levels of AHH and genetically-regulated expression of cancer using "cancer-free" and "cancer-prone" families.

Major Findings: Studies have been initiated using the standard fluorometric assay for AHH determination and coded samples of blood. Twelve ml samples were shipped in thermos bottles enclosed in polystyrene boxes to keep the samples above 15°C. If received within a 24-hour period, the lymphocytes can be isolated, cultured and assayed for AHH activity. Data indicate that while duplicate samples from one individual gave similar results if assayed on the same day, samples from the same individual assayed on different days could give quite disparate results. The major problem seems to be non-uniform mitogen-activated lymphocyte populations from which to assay. This mitogen-activation step is affected by such culture conditions as types, sources, lots, and concentrations of mitogens, lots of fetal calf serum, kinds of culture vessels, and presence of autologous human serum in the medium. Even with this more uniform cell population from which to assay, it is proposed that at least two assays, the fluorometric assay measuring conversion of BP to 3-OH BP (at pH 8.5) and the radiometric assay measuring conversion of BP from an organic-soluble to aqueous-soluble form, may be required to positively assign particular individuals a specific AHH activity.

Significance to Biomedical Research and the Program of the Institute: The determination of the biochemical basis of at least certain forms of cancer in man is of obvious significance to biochemical research and the program of the Institute. AHH levels may not only be a major determinant in man's ultimate susceptibility to chemically-induced cancers, but also auger the possibility of new approaches to the control of cancer.

Proposed Course: Four courses of action will be pursued--(1) standardization of the assay, including establishment of a reproducible constitutive AHH activity using either particular lots of fetal calf serum or other inducers, such as phenobarbital and estradiol; (2) completion of a blind study to the satisfaction of the project officer; (3) comparison of the fluorometric and radiometric assays; and (4) assay samples from the first "cancer-prone" family.

Date Contract Initiated: September 9, 1974

Current Annual Level: $99,900

MIDWEST RESEARCH INSTITUTE (NO1-CP-33387)

Title: Organic Synthesis of Cold and Tritiated Polycyclic Hydrocarbon Derivatives

Contractor's Project Director: Dr. James F. Engel

Project Officer (NCI): Dr. James K. Selkirk

Objective: The overall objective of this program is the preparation of NCI-selected compounds by unequivocal methods to produce gram quantities of high-purity and well-characterized materials.

Major Findings: To date, approximately 40 derivatives of 5 parent hydrocarbons have been prepared, including several with tritium labels. The hydrocarbons under study are benzo[a]pyrene; 3-methylcholanthrene; 7,12-dimethyl-benz[a]anthracene; and dibenz[a,h]anthracene. The products prepared thus far have included K-region and non-K-region phenols, quinones, epoxides, dihydrodiols, dialdehydes, and alkyl- and hydroxyalkyl-substituted derivatives.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the relationship between the formation of specific metabolites of polycyclic aromatic hydrocarbons and the induction of cancer, necessitates the availability of authentic samples of probable metabolites. It would be virtually impossible for a laboratory lacking specific synthetic experience to synthesize any one of these compounds for a specific experiment. This contract allows the National Cancer Institute to provide compounds for pertinent experiments in chemical carcinogenesis which could not be carried out otherwise.

Date Contract Initiated: June 15, 1973

Current Annual Level: $197,390

NEW YORK UNIVERSITY (NO1-CP-33279)

Title: The Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions

Contractor's Project Director: Dr. Claudio Basilico

Project Officer (NCI): Dr. John P. Bader

Objectives: (1) isolation and characterization of temperature-sensitive (ts) mutants of somatic animal cells; and (2) use of these mutants to study mutation mechanisms in somatic animal cells, and the effect of specific biochemical lesions on the regulation of cell growth and division.

Major Findings: Isolated are over 70 ts mutants of the hamster cell line BHK 21. These mutants exhibit low reversion and low leakiness. Their ts growth is due to a variety of defects as shown by genetic and biochemical analysis. All mutations tested are recessive. In five cases, the ts mutation does not appear to be X-linked, but the available evidence shows that these mutations are chromosomal in nature. Complementation tests have assigned the mutants tested so far to 13 complementation groups, with a few groups containing more than one mutant. Two ts mutants have been characterized to a satisfactory extent: the ts422E mutant cannot grow at 39° because of a defect in the processing of ribosomal RNA precursors, leading to a block in the production

of 28S ribosomal RNA. This defect is probably due to the presence of a ts ribosomal protein. The ts422E mutation can be directly complemented in intra- and inter-specific hybrids. However, the complementation between ts422E and human cells is imperfect, leading only to a partial restoration of the ts defect. Mutant tsAF8 becomes arrested at 39° in the G1 phase of the mitotic cycle. The phenotypic expression of this mutation is modified upon transformation by polyoma virus. Some of the ts BHK mutants are also ts in their capacity to support the multiplication of adenovirus 2. The block to viral multiplication appears related to the specific cell function affected in these mutants at high temperature.

Significance to Biomedical Research and the Program of the Institute: Cancer cells have altered regulation of proliferation and differentiation. Conditional lethal ts mutations have proven to be extremely useful for a variety of studies with microorganisms and their usefulness to further our understanding of somatic animal cells and their regulatory mechanisms is beginning to be demonstrated. Such mutants, in particular those affected in macromolecular synthesis, cell cycle regulation, and possibly membrane formation, are extremely valuable in studies of carcinogenesis. They should provide an understanding of the cell regulatory mechanisms and of their breakdown during neoplastic transformation, which is basic to our understanding of cancer.

Proposed Course: The work for the next years is aimed at the following points: (1) the isolation and study of additional ts mutants, with emphasis on specific classes of mutants, DNA negative or cell cycle mutants; (2) the further characterization of the mutants already obtained, both from the biochemical and genetic point of view; and (3) the use of some of the mutants for specific experiments such as the investigation of the relationship between ribosomal RNA synthesis and cell division, the effect of viral transformation on the characteristics of the cell cycle, and the study of what determines cell transition from a growing to a resting state. The study of the host cell functions required for adenovirus multiplication will be continued, and we plan to isolate viral mutants which overcome the cellular block with the hope that they may possess nonsense mutations.

Date Contract Initiated: June 26, 1971

Current Annual Level: $111,960

OHIO STATE UNIVERSITY (NO1-CP-43248)

Title: Research on Preparation of Polymers Containing Carcinogenic Moieties

Contractor's Project Director: Dr. Melvin Newman

Project Officer (NCI): Dr. James K. Selkirk

Objective: To develop affinity chromatography supports for the isolation and purification of aryl hydrocarbon hydroxylase (AHH)

Major Findings: (1) Improved synthesis of 1,2-maphthalic anhydride, A, an important intermediate for synthesis of benz[a]anthracene compounds.

(2) Synthesis of 10-Chloro-7, 12-dimethylbenz[a]anthracene,B. This compound is needed for eventual conversion into 10-hydroxymethyl-7,12-dimethylbenz[a]-anthracene, C, and10-diazomethyl-7,12-dimethylbenz[a]anthracene, D. It is hoped that B and C compounds will be converted to a polymer to fulfill the objective of this research, namely, to see if polymers containing carcinogenic compounds attached by nonbiodegradable linkages will produce cancer.

Small amounts of B have been prepared. The synthesis proved to be unusually difficult but now we know how to do it. The preparation of large amounts of B, C, and D is now being done. These syntheses have enabled preparation of 10-methylbenz[a]anthraquinone, E, from which routes to C and D can be envisioned. These routes are now under study.

(3) The synthesis of 2,7,12-dimethylbenz[a]anthracene, F, was accomplished early in the program to ascertain whether attachment of a polymer chain at the 2-position would be suitable for the objectives of the contract. Tests at the MacArdle Laboratory for Cancer Research have shown, however, that F was not carcinogenic. Hence, further work on analogs of F has been discontinued.

Significance to Biomedical Research and the Program of the Institute: Since AHH is closely involved in the detoxification and, possibly, in the activation of carcinogenic polycyclic hydrocarbons, it is important to define clearly the pathway of metabolism by purification of the enzymes.

Proposed Course: To prepare polymers in which benzo[a]pyrene (BP) is connected to a polymer. A quantity of 6-hydroxymethylbenzo[a]pyrene, G, have been made for reaction with p-chloromethylstyrene to obtain a monomer, H. Alternately attempts are in progress to react poly-p-chloromethylstyrene with G to obtain a polymer suitable for testing for carcinogenic activity.

Date Contract Initiated: January 11, 1974

Current Funding Level: $31,100

OMNI RESEARCH INC. (NO1-CP-33386)

Title: Organic Synthesis of Cold and Tritiated Polycyclic Hydrocarbon Derivatives

Contractor's Project Director: Dr. Owen H. Wheeler

Project Officer (NCI): Dr. James K. Selkirk

Objective: To prepare tritium labeled derivatives of 7,12-dimethylbenz[a]-anthracene

Major Findings: Methods have been developed for the synthesis of 7-hydroxymethyl-12-methylbenz[a]anthracene; 12-hydroxymethyl-7-methylbenz[a]-anthracene; 7,12-dimethylbenz[a]anthracene; and cis-5,6-dihydro-5,6-dihydroxy-7,12-dimethylbenz[a]anthracene.

Significance to Biomedical Research and the Program of the Institute: These compounds are being synthesized to provide substrates for cell and enzyme studies pertaining to the induction and activity of the aryl hydrocarbon hydroxylase enzyme system.

Proposed Course: The methods which have been developed will be used to prepare the compounds in tritium labeled form starting with tritiated 7,12-dimethylbenz[a]anthracene.

Date Contract Initiated: June 29, 1973

Current Annual Level: $27,500

ONTARIO CANCER INSTITUTE (NO1-CP-43331)

Title: Isolation, Propagation and Search of Mutant Vertebrate Cells

Contractor's Project Director: Dr. Louis Siminovitch

Project Officer (NCI): Dr. John P. Bader

Objectives: (1) Isolation of cell lines carrying mutations affecting key cell functions such as DNA replication or mitosis, or key cell structures such as the plasma membrane. (2) Biochemical and physiological characterization of the mutant lines. (3) Examination of the properties of hybrid cell lines obtained by fusion of cells of one mutant line with other normal or mutant lines, for complementation analysis and possible mapping. (4) Extension of the techniques, as they are developed, to a variety of cell types chosen not only on the basis of their suitability for studies of the regulation of cell multiplication and differentiation, but also for other purposes, such as the detection of environmental carcinogens and mutagens.

Major Findings: The temperature-sensitive conditional-lethal mutant (ts) of CHO cells (called tsH1), is recessive and the lesion has been shown to involve a temperature-sensitive leucyl-tRNA synthetase. A new selection was developed for temperature-sensitive mutations involving lesions in protein synthesis. Recessive mutants involving asparaginyl-tRNA synthetase, as well as leucyl-tRNA synthetase, were successfully isolated using these selections. Mutants of CHO cells resistant to α-amanitin show an altered RNA polymerase II. The marker behaves codominantly in hybrids between resistant and wild-type cells. Mutants resistant to histidinol and tyrosinol have been selected. Selection for resistance to colchicine leads to mutants with altered permeability to the drug, and which show collateral resistance to other drugs. Concanavalin (Con A) resistant cells show increased sensitivity to dibutyryl cAMP and temperature. Phytohaemagglutinin (Pha) resistance leads to mutants which show increased sensitivity to Con A, and which are recessive in somatic cell hybrids.

Mutants resistant to agglutinins from wheat germ ricinis hominis and lens culinaris have also been selected. Preliminary results have been obtained that the Pha-resistant cells are missing a membrane glycosyl transferase. Mutants resistant to ouabain have been isolated in human diploid cells. Rat myoblast mutants resistant to α-amanitin have been shown to possess a toxin-resistant RNA polymerase II. In such mutants RNA polymerase II activity is regulated independently of RNA polymerase I and is not expressed constitutively. Myogenesis in the rat myoblast line is affected by agents which perturb cAMP metabolism. Mutants resistant to the cytostatic action of dibutyryl cAMP have been isolated and partially characterized.

Significance to Biomedical Research and the Program of the Institute: Cancer may be regarded as a disease in which cells are genetically altered such that their regulation of cellular multiplication and differentiation is defective. Temperature-sensitive mutations affecting key functions involved in cell growth, and in particular DNA replication and mitosis are of specific interest in respect to obtaining an understanding of the breakdown of regulation during carcinogenesis. Suitably characterized cell lines are useful not only for studies of cellular regulatory processes, but for other purposes as well. One example is the use of cell culture systems for the detection of environmental carcinogens or mutagens. In addition, studies on the genetic basis of drug resistance are of special interest in respect to chemotherapy. For example, our finding that mutation to resistance to colchicine and to Con A engenders collateral changes in sensitivity to other drugs must be considered in contemplation of multiple drug trials.

Proposed Course: To continue with the studies as outlined above.

Date Contract Initiated: January 1, 1972

Current Annual Level: $178,000

POLYSCIENCES, INC. (NO1-CP-23245)

Title: Optimizing Electrophoretic Separation of Proteins with New Hydrogels

Contractor's Project Director: Dr. B. David Halpern

Project Officer (NCI): Dr. Andrew C. Peacock

Objectives: To develop hydrogel systems specifically suitable for gel electrophoretic separation of biological macromolecules related to carcinogenesis studies. This study is aimed at achieving the gel properties such as (a) large pore size to permit the study of large molecules, (b) desirable associative effects to improve the specificity and resolution, and (c) greater elutability of substances from gels.

Major Findings: Bifunctional polyethylene glycol diacrylate (PEGDA 20,000) gave gels of large pore size whereas hexafunctional polyethylene glycol hexaacrylate 20,000 (PEGHA 20,000) gave tighter gels as compared to acrylamide-methylenebisacrylamide (AA-MBA) gels of the same solid content. PEGDA 20,000

(1.5%) agarose (0.5%) composite gels showed intercept MW of 1.0×10^8 daltons, indicating a gel system of extremely large pore size. E. coli, rat liver and yeast tRNAs separated into unique patterns of bands in 20% AA-MBA (19:1) slab gels containing 6% AA-MBA (19:1) stacking gel. E. coli, rat liver and yeast tRNAs separated into 23 bands, 24 bands and 19 bands, respectively. Incorporation of a cationic monomer (MCC) or a Zwitterionic monomer, at 0.03% or 1%, respectively, in the stacking gel resulted in much better and more distinct separation. The results indicate that charge effect is also an important factor in tRNAs separation by gel electrophoresis.

Significance to Biomedical Research and the Program of the Institute: The findings obtained by the above experiments are of considerable importance to the program. The large pore gels are valuable for electrophoretic separations of very large bio-molecules. The high degree of tRNA separation will contribute to the full exploration of tRNAs. Ability to more exactly separate cell constituents will aid other researchers studying cancer cause and effect relationships.

Proposed Course: The large pore gels from bifunctional PEGDA 20,000 will be examined for their practical use for larger biological sub-units such as polysomes and viruses separations. Experiments will be conducted to recover specific tRNA and aminoacylation using purified enzyme will be carried out to prove that the high degrees of separation are free of shearing and other artifacts.

Date Contract Initiated: May 17, 1974

Current Annual Level: $62,609

ROCHESTER, UNIVERSITY OF (NO1-CP-45611)

Title: Mammalian Cell Transport Systems: (2) Purification of Plasma Membrane Transport Systems

Contractor's Project Director: Dr. John Donald Hare

Project Officers (NCI): Dr. Tsuyoshi Kakefuda
Dr. Ann E. Kaplan

Objective: To identify and characterize membrane proteins whose function is to transport substrates across the cell membrane in normal and neoplastic cells. The approach used is to synthesize analogs of several classes of substrates that would function as photoaffinity probes to label putative carrier proteins and follow their isolation.

Major Findings: Initial studies have been directed at the synthesis of phenylalanine photoprobes with the ability to be recognized by the transport system which handles phenylalanine and to be activated to bind covalently to macromolecules for which the probe shows preferential binding. Many of the steric constraints have been recognized and analogs synthesized which carry photosensitive groups. It has been possible to inactivate irreversibly several

transport systems with one probe. Molecular modifications that will increase the specificity of such a phenylalanine analog are being investigated now.

Significance to Biomedical Research and the Program of the Institute: The structure and function of the cell membrane has come to be recognized as an important intermediate in the mechanism by which a cell in the tissue and organs of higher organisms, including the human, is regulated both in cell function and cell division. The detailed molecular mechanisms by which nutrient substrates are taken up by the cell has not yet been fully elucidated. Furthermore, the role played by modification in the various substrate transport systems in the process of carcinogenesis is under active investigation. Therefore, studies directed at an understanding of the transport system in mammalian cells and changes in their structure or function with carcinogenesis will contribute to the knowledge required to document these changes and potentially to take advantage of the changes to develop specific drugs to control cancer cells.

Proposed Course: To synthesize a series of photoactivable analogs of amino acids and nucleosides with which to label macromolecules associated with the transport systems of mammalian cells, thereby allowing their identification and isolation from among the variety of proteins in the cell membrane.

Date Contract Initiated: June 26, 1974

Current Annual Level: $110,000

TEXAS, UNIVERSITY OF (At Austin) (NO1-CP-33384)

Title: Growth of Soil Bacteria on Polycyclic Hydrocarbons

Contractor's Project Director: Dr. David T. Gibson

Project Officer (NCI): Dr. James K. Selkirk

Objectives: (1) To explore the possibility of the formation of chemical carcinogens by the action of soil bacteria on polycyclic hydrocarbon precursors; (2) to isolate and identify oxygenated metabolic products formed from polycyclic hydrocarbons by bacteria; (3) to obtain mutant strains of bacteria that accumulate significant amounts of oxygenated products for supply to the scientific community for in vivo and in vitro studies in carcinogenesis; and (4) to develop synthetic routes for the formation of arene oxides from microbiologically-produced cis-dihydrodiols.

Major Findings: A strain of Beijerinckia that grows with biphenyl or phenanthrene as sole source of carbon was isolated from a polluted stream. Cells of this organism after growth on either phenanthrene or biphenyl oxidized both benzo[a]pyrene and benz[a]anthracene to polar products. A mutant strain of this organism was obtained that oxidized biphenyl and phenanthrene to cis-2,-3-dihydroxy-2,3-dihydrobiphenyl and cis-3,4-dihydroxy-3,4-dihydrophenanthrene respectively. This mutant organism oxidized benzo[a]pyrene to a compound that

has been isolated and identified as cis-9,10-dihydroxy-9,10-dihydrobenzo[a]-pyrene. The structure was determined by acid catalyzed dehydration to yield 9-hydroxybenzo[a]pyrene and an analysis of its proton magnetic resonance spectrum. The same organism oxidized benzo[a]anthracene to three different dihydrodiols which were separated by high pressure liquid chromatography. These products have been tentatively identified as cis-1,2- 7,8- and 9,10-dihydroxydihydrobenzo[a]anthracenes.

This bacterium appears to attack polycyclic hydrocarbons at positions similar to those reported for mammalian microsomes. However, the mode of oxidation is different, and at this time it appears that the K-regions are not subject to metabolism.

Significance to Biomedical Research and the Program of the Institute: Polycyclic aromatic hydrocarbons have been shown to occur in air, water and food. Thus they come into contact with all forms of life. At this time there are no detailed reports on the metabolism of these compounds by bacteria. This report documents for the first time the bacterial oxidation of benzo[a]pyrene and benz[a]anthracene to dihydrodiol derivatives. These dihydrodiol derivatives are formed at the same positions as those produced by the microsomal oxidation of benzo[a]pyrene and benz[a]anthracene. However, the microbially-produced dihydrodiols have a cis stereochemistry which is in direct contrast to the trans-dihydrodiols produced by mammals. These results indicate that bacteria do not form electrophilic arene oxides that have been implicated as the causative agents in chemical carcinogenesis.

Proposed Course: To proceed with the isolation and identification of the metabolic products formed from polycyclic hydrocarbons by microorganisms. To attempt to produce sufficient quantities of cis-dihydrodiol derivatives for the synthesis of several polycyclic hydrocarbon derivatives which will be made available to the scientific community through the Carcinogenesis Program.

Date Contract Initiated: June 25, 1973

Current Annual Level: $57,915

TEXAS, UNIVERSITY OF (at Houston) (NO1-CP-45604)

Title: Studies on Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans

Contractor's Project Director: Dr. Charles R. Shaw

Project Officers (NCI): Dr. Harry V. Gelboin
Dr. Friedrich J. Wiebel

Objectives: (1) To simplify and improve the reproducibility of the assay for measuring aryl hydrocarbon hydroxylase (AHH) in human lymphocytes; (2) to measure the activity and inducibility of AHH in a normal human population;

(3) to determine the levels of AHH activity and inducibility in cancer patients, particularly those with bronchogenic carcinoma; and (4) to measure AHH levels in families in an effort to understand the genetic control of this enzyme.

Major Findings: The major effort during the initial contract period was to determine the extent and causes of variability of AHH activity in short-term lymphocyte cultures. A double-blind study was carried out, involving 23 individuals. Fresh blood samples were provided by another laboratory and identified to us only by code numbers. In most instances each volunteer donated several blood samples at one time, and this procedure was repeated at least one other time during the five-month period of study. A total of 275 blood samples were processed.

Variability within multiple samples drawn on the same day was relatively low. However, variability within samples drawn on different days was, for some individuals, significantly high. Some variation can be attributed to difference in media, particularly to components such as fetal calf serum and mitogens. The possibility cannot be ruled out, however, that variability may also reflect day-to-day differences in the peripheral lymphocyte population.

AHH inducibility has been measured in over 200 normal individuals. The distribution of inducibility resembles a log normal curve. This may be an indication of a lack of precision of the analysis or that AHH inducibility is a polygenically determined trait.

Blood samples have been obtained from approximately 75 lung cancer patients. The lymphocytes obtained from nearly half these patients did not respond to mitogen stimulation, and about another fourth responded poorly. The distribution of AHH inducibility in the remaining lung cancer patients gives the impression of a difference from the normal population, but due to the variability of the method, the differences between lung cancer patients and the normal population are not statistically significant.

Significance to Biomedical Research and the Program of the Institute: This project is ultimately concerned with understanding the relationship between AHH inducibility and susceptibility to cancer, particularly bronchogenic carcinoma. The studies will likely lead to the elucidation of particular chemical carcinogens in the case of certain cancers and may point to directions for specific preventive procedures. Additionally, the studies will doubtless lead to better understanding of the mechanisms of carcinogenesis in some of these tumors.

Proposed Course: The present effort is directed toward solving the problem of variability. A major source of variation is in the processes of blastogenesis and growth, rather than in the assay for enzyme activity. Expressing specific activity in terms of number of cells does not appear to be satisfactory. Activity expressed per milligram of protein leads to more reproducible results. Other ways of expressing specific activity will be evaluated in hopes of reducing the variability now observed in the assay. AHH inducibility in a large normal population will be measured with the use of an improved assay. Patients with various types of cancer will be studied to see if a relationship

exists between the AHH system and the occurrence of cancer.

Date Contract Initiated: August 15, 1974

Current Annual Level: $95,400

TEXAS, UNIVERSITY OF (M.D. Anderson Hospital and Tumor Institute) (NO1-CP-43270)

Title: Non-Histone, DNA Binding Proteins from Normal Rat Liver and Chemically-Induced Rat Hepatomas

Contractor's Project Director: Dr. Lubomir S. Hnilica

Project Officers (NCI): Dr. Andrew Peacock
Dr. C. Wesley Dingman

Objectives: To isolate and characterize practical amounts of tissue-specific DNA binding nonhistone chromosomal proteins. It is anticipated that these proteins function in gene regulatory capacity. The strict immunospecificity of the DNA-binding proteins will be utilized for the detection of neoplastic cells in tissue sections and biopsies.

Major Findings: A fraction(NP) of chromosomal nonhistone proteins was isolated from rat liver and hepatoma. These proteins bind preferentially to homologous, double-stranded DNA ($Km5 \times 10^{-9}$) and the complexes are immunologically tissue specific. The NP proteins consist of two major and several minor components. One component is strongly acidic; the other, moderately basic. Hepatocarcinogenesis changed the immunospecificity of the NP-DNA complexes from that typical for normal liver to a new type, characteristic for hepatomas and other malignant tumors. By in vitro reconstitution of DNA and chromatin protein fractions, the hepatoma immunospecificity could be transferred to liver chromatin by substituting the hepatoma NP proteins for the liver NP fraction.

Significance to Biomedical Research and the Program of the Institute: The process of carcinogenesis frequently changes the transcriptional controls of individual genes. This leads to the expression of a new cellular phenotype, characteristic for the transformed cell. Work supported by this contract demonstrated that carcinogenesis changes the immunological and biochemical specificity of a small group of chromosomal nonhistone proteins (NP). This change appears to be common to all tested experimental neoplasms and points to a change in the transcriptional control of the transformed cell. Since the change in the immunospecificity of the NP-DNA complexes is characteristic for malignant neoplasms which share a common antigenic feature (i.e. that of cancerous cells) it should be possible to utilize such specificity for the immunochemical detection of cancerous cells in tissue sections and biopsies.

If the tissue-specific chromosomal nonhistone proteins are part of the cellular gene-regulation mechanisms, detailed knowledge of gene-regulatory

macromolecules may lead to new biochemical techniques for the control or prevention of carcinogenesis.

Proposed Course: The chromosomal nonhistone proteins with affinity for homologous DNA will be further purified, fractionated and characterized. The involvement of these proteins (NP fraction) in malignant transformation of cells will be investigated. Preliminary experiments indicate that the NP-DNA complex may be the site of the interaction of chemical carcinogens with cellular DNA. The immunospecificity of NP-DNA complexes from malignant neoplasms will be further tested and utilized for the in situ detection of transformed cells (horse radish peroxidase and diamino benzidine reaction). It appears that this technique may be developed into a rapid method for differentiation of normal and cancerous cells in histological sections.

Date Contract Initiated: June 29, 1972

Current Annual Level: $93,710

WEIZMANN INSTITUTE OF SCIENCE (NO1-CP-33220)

Title: Alterations in Translation of Genetic Messages Induced by Viruses and Carcinogens

Contractor's Project Director: Dr. Michel Revel

Project Officers (NCI): Dr. Harry V. Gelboin
Dr. Dolph Hatfield

Objectives: (1) To investigate the possibility that carcinogens alter the translational components of the cell resulting in a change in the expression of the genetic messages; and (2) to identify viral molecular elements which may be related to transformation and elucidate the relationship of their mechanism of action to chemical carcinogenesis.

Major Findings: (1) A correlation was observed between the deficiency of Y-base in the anticodon loop of $tRNA^{phe}$ and tumor cells which may serve as a new screening method for human cancer. (2) Purified messenger RNA coding for virus proteins was isolated from cells infected by SV40 and translated in cell-free extracts. The two late classes of SV40 mRNA (16 and 19s) were separately purified and found to code for different products: SV40 16s RNA directs the synthesis of the major capsid protein VP-1, while SV40 19s RNA codes for a novel non-virion protein called the X polypeptide. (3) Virus-specific RNA isolated fromSV40 transformed cells directs the synthesis of a product with similar molecular weight to the polypeptide isolated from SV40-infected cells by immunoprecipitation with hamster anti-T serum. (4) A new procedure was developed to isolate viral mRNA by its hybridization to immobilized viral DNA. This has potential use in screening for the presence of viral sequences in tumor cells.

Significance to Biomedical Research and the Program of the Institute: The change in expression of genetic information as a function of carcinogen

induced cell transformation may be viral or cellular in origin. The elucidation of the molecular mechanism by which carcinogens bring about this change is important for a better understanding of tumorigenesis. From these studies potential screening devices for the diagnosis of human cancer may emerge as well as new possibilities for cure and prevention of the disease.

Proposed Course: (1) Further analysis of different tumors for the level of Y-base in $tRNA^{Phe}$ in an attempt to establish a screening method for cancer. The possibility that carcinogen modified mRNA or tRNA result in miscoding will be explored. (2) Viral specific mRNAs will be further isolated and identified, mainly mRNA coding for SV40 Tantigen and C-type viral MRNAs (3) Study the mechanism by which carcinogens induce Polyoma virus production. (4) Study the possibility that carcinogens induce expression of C-type viral RNA.

Date Contract Initiated: June 1, 1973

Current Annual Level: $239,400

WEIZMANN INSTITUTE OF SCIENCE (NO1-CP-02217)

Title: Study of the Role of Enzyme Induction and Chemical Carcinogenesis

Contractor's Project Director: Dr. Leo Sachs

Project Officer (NCI): Dr. Harry Gelboin

Objective: To clarify the role of polycyclic metabolism in carcinogenesis.

Major Findings: (1) Cell Mediated Mutagenesis of Mammalian Cells with Chemical Carcinogens--Chemically non-reactive carcinogens have to be metabolized by cellular enzymes to exert their biological effects including mutagenicity. Chinese hamster V79 cells can be efficiently mutated from 8-azaguanine susceptibility to resistance; but these cells do not metabolize polycyclic hydrocarbons and were, therefore, not mutated by these compounds. A system of cell mediated mutagenesis with carcinogenic hydrocarbons has been developed, by co-cultivating V79 cells with lethally irradiated rodent cells that can metabolize the carcinogens. Cell-mediated mutagenicity was obtained with the carcinogenic hydrocarbons 7,12-dimethylbenz[a]anthracene, benzo[a]pyrene, 3-methylcholanthrene, 7-methylbenz[a]anthracene and dibenz[a,h]anthracene and there was no mutagenicity with the non-carcinogenic hydrocarbons pyrene, chrysene, phenanthrene and benz[a]anthracene. The degree of mutagenicity was, therefore, related to the degree of carcinogenicity and our method detected mutagenicity with 0.01 µg/ml. Cell-mediated mutagenesis with human cells, should provide a useful system to test for environmental chemical hazardous to humans that have to be metabolically activated.

(2) Regulation of Cell Susceptibility to Carcinogenic Polycyclic Hydrocarbons by Cyclic AMP and its Genetic Control--The metabolism and cytotoxic effect of the carcinogenic hydrocarbons benzo[a]pyrene, 20-methylcholanthrene and 7,12-dimethyl-benz[a]anthracene can be regulated in some cell types by treating

the cells with dibutyryl cyclic AMP (dc AMP) or with compounds such as aminophylline, theophylline and prostaglandin E_1, that increase the cellular level of cAMP.

The amount of BP metabolism in 27 different cell types from various mammals including humans, ranged from less than 0.1 µg to 2.3 µg metabolized BP per 10^6 cells. Treatment of these different cell types with aminophylline gave either an increase in BP metabolism, an induction of metabolism in cells that did not metabolize without aminophylline, or no induction after treatment with aminophylline and dcAMP. The existence of responding and non-responding cell lines indicates that the regulation of the level of polycyclic hydrocarbon metabolism by dcAMP is genetically controlled. The induction of metabolism in cells that did not metabolize without aminophylline resulted in the conversion of cell resistance to cell susceptibility to the cytotoxic effect of BP. Treatment with dcAMP and aminophylline can, therefore, be used to increase the sensitivity of screening tests for chemical carcinogens.

Significance to Biomedical Research and the Program of the Institute: An understanding of these biochemical processes is necessary to understand carcinogenesis in humans.

Proposed Course: To further clarify the relationship between the presence of the enzyme AHH enzyme system, the metabolism of carcinogenic hydrocarbons, and malignant cell transformation.

Date Contract Initiated: May 26, 1970

Current Annual Level: $145,000

YALE UNIVERSITY (NO1-CP-55673)

Title: Selection and Propagation of Haploid and Partial Monosomic Cell Lines

Contractor's Project Director: Dr. Frank H. Ruddle

Project Officer (NCI): Dr. John P. Bader

Objectives: To isolate monosomic, partial monosomic and haploid cells from diploid or near diploid populations of mouse cells. Both genetic markers and cytogenetic analysis will be used to detect and verify the presence of the desired cell types.

Major Findings: Cell lines from embryos carrying duplications and deficiencies due to translocations carried in the heterozygous state by the parents, and cell lines with diploidkaryotypes to be used in experiments on loss of chromosomes have been developed. Such procedures will yield monosomic lines.

Significance to Biomedical Research and the Program of the Institute: The derivation of well-characterized monosomic and hapliod cells from mouse will provide research materials with which to define the genetic factors which influence cellular transformation in vitro and tumor progression in vivo.

The presence of single genetic determinants in such cells will make them useful in man x mouse somatic hybrids which can be used to define homologous genetic units in man by a complementation assay. Such cell lines can be used to study the relationship of gene dosage and the mechanism of action of carcinogens and mutagens.

Proposed Course: To develop diploid cell lines, use them in chromosome elimination experiments, and to continue studies on cultivation of spermatids and hybrids of spermatids with other cells. This experimental series has just begun.

Date Contract Initiated: December 18, 1973

Current Annual Level: $99,500

SUMMARY REPORT

COLON CANCER SEGMENT

July 1, 1974 through June 30, 1975

The objective of the Colon Cancer Segment is to identify the etiologic factors responsible for colon cancer in humans. It combines laboratory studies with epidemiological observations in order to determine etiological elements inducing colon carcinogenesis and those factors which are involved in its control. The major part of the program is based on epidemiological evidence relating diet, intestinal microflora, and colon cancer. In particular, the relationship of beef consumption is under investigation both directly and in related experiments.

Two epidemiological surveys are supported to determine whether changes in diet can be correlated with the increasing intestinal cancer incidence among blacks. One study by the Kaiser Foundation Research Institute (NO1-CP-33215) is based on the San Francisco Bay area. Interviews with cancer patients are matched by similar interviews with persons of the same age and sex hospitalized for some disease unrelated to the gastrointestinal tract. Preliminary analysis is in progress for case-control differences in dietary practices. To date no significant difference has been observed. The Medical College of Georgia (NO1-CP-43282) is initiating a similar study based on admissions to nine Atlanta hospitals. These surveys should indicate whether the correlation between a diet high in meat and the occurrence of colonic tumors can be confirmed in another population at increasing risk to this disease.

The Naylor Dana Institute of the American Health Foundation (NO1-CP-43378) is studying the effect of meat proteins and fat in the diet of germ-free and conventional rats exposed to known carcinogens. The contractor has utilized intrarectal instillation of the carcinogen in some cases and has followed development of the lesions in situ by endoscopy. Two bile acids, either cholic and taurodeoxycholic, have enhanced tumor production by N-methyl-N'-Nitro-N-nitrosoguanidine (MNNG). In a study of two generations of rats on high or low fat diets and Purina Laboratory Chow, 1,2-dimethyl-hydrazine (DMH) combined with a high fat diet (20%) induced more tumors than did the carcinogen plus a low fat (5%) diet. DMH administered by the subcutaneous route, produced about one-fifth as many tumors in germ-free rats as in conventional rats. Large bowel tumors were induced in strain 2 guinea pigs by MNNG and methylnitrosourea.

Several investigators are determining what differences, if any, can be detected in the microflora of the gut of persons from populations at different risks of colonic cancer. At the Anaerobe Laboratory of Virginia Polytechnic Institute and State University (NO1-CP-33334) bacteriological studies have now been completed on individuals from four different population groups. Statistical comparisons are underway. From the study of 20 Japanese-Hawaiians cohort members of the Cancer Study Group in Hawaii who are free of intestinal tumors, 113 distinct types of bacteria were identified among the

1,147 isolations made; over half had not been identified previously. This Group observed that in small samples of healthy North Americans or Japanese-Hawaiians about 31 percent converted cholesterol to a small degree into coprostanol and coprostanone in the large intestine; among a group of individuals with a history of adenomatous polyps over 60 percent were "low convertors." Cholesterol was found more extensively degraded in the feces of rural South Africans of the Tswana tribe of whom only 5 percent converted less than half of the available cholesterol. These studies will be extended to other population groups at low and high risk. At the University of California at Los Angeles (NO1-CP-33380), the flora of individuals on Japanese diet or a low meat or meat-free diet (Seventh Day Adventists) is being compared with the flora of control groups on a typical western diet. This contractor is also comparing the flora of persons found by colonoscopy to bear intestinal polyps with persons free of tumors. The University of Missouri School of Medicine (NO1-CP-33335) staff has just completed the laboratory aspects of a study of fecal flora and chemistry of subjects on various diets. A group of ten males was placed successively on a normal western diet, a vegetarian, and high beef diet, and finally on their normal diet. Fecal samples were collected and studied during the fourth week of each diet period. The data are being analyzed; at present no marked dietary effect has been noted.

Another study at the University of California at Los Angeles (NO1-CP-43290) revealed that man, unlike rats, mice or monkeys, has no intestinal microflora peculiar to the intestinal mucosa. A study was initiated recently to determine the effect of diet dependent changes in enzyme activity of the rat fecal flora. Similar studies are to be done with the enzymes of human intestinal organisms. Activity of β-glucuronidase increased with a change from a cereal to a meat diet while that of β-glucosidase decreased. Among the enzymes to be studied are N-dehydroxylase, N-hydroxylase, nitro-reductase, aryl sulfatase, and azo-reductase.

At Memorial Hospital for Cancer and Allied Diseases (NO1-CP-43366), studies are in progress both to develop indices for a screening program for human biopsy samples and for persons with familial polyposis and Gardner's syndrome and their relatives, and to try to determine the genetic defect leading to colon cancer in the inherited diseases. The indices now being used in the screening program are physiological factors, such as the ability of neoplastic cells to grow in low (1%) serum, and changes in the control of the cell growth cycle, and biochemical factors, based on the continuous appearance of enzymes needed for DNA synthesis in cells which have normally lost this synthetic ability in the colonic mucosa. Current studies on the genetic defect include the interaction in chromatin between the non-histone proteins and DNA, and the investigation of DNA damage and repair with the Brookhaven National Laboratory. The group at Memorial Hospital has also developed a model system in rodents for the study of colon cancer, and a method for growing biopsy samples in immunodeficient mice, which is important for histological identification and for the screening program analyses.

CONTRACT NARRATIVES

COLON CANCER SEGMENT

July 1, 1974 through June 30, 1975

AMERICAN HEALTH FOUNDATION (NO1-CP-43378)

Title: Studies in Colon Carcinogenesis

Contractor's Project Directors: Dr. John H. Weisburger
Dr. Ernst L. Wynder
Cr. Bandaru Reddy

Project Officer (NCI): Dr. Sidney J. Silverman

Objectives: Factors bearing on cancer causation in the colon are investigated in animal models. Specifically, the following points are under investigation: the mechanisms are determined by which the high meat protein and fat diet, known from epidemiologic data to be associated with a high colon cancer risk, is translated into specific carcinogenic, cocarcinogenic and/or promoting factors which affect the large bowel. Suitable animal models of colon carcinogenesis are developed in bacteriologically-defined and controlled systems. The effect of dietary factors, especially fats and protein, as modifiers of colon carcinogenesis, is studied. The cocarcinogenicity of selected cholesterol bile acid and tryptophan metabolites is determined. Pure strains of the major groups of intestinal microflora are isolated from high and low risk populations, and their enzymatic capability to degrade acid and neutral steroid into potentially carcinogenic or cocarcinogenic compounds is studied.

Major Findings: A bioassay system for colon carcinogens involving intrarectal (IR) administration of suspected agents in mice, rats, and guinea pigs was further developed. Methylnitrosourea (MNU) yields colon cancer faster than methyl-nitro-nitrosoguanidine (MNNG), but it also gives rise to leukemia and, in mice, to lung tumors. IR MNNG yields more tumors in rats on chow than on a semi-synthetic diet. Rats on a vitamin A-deficient diet are less sensitive than controls. Endoscopic examination of rats has been perfected to detect early colon cancers, which permits studies in pathogenesis without serial killing. With IR MNU in mice, more frequent small doses yielded more colon cancers than fewer large doses.

Taurodeoxycholic and lithocholic acid given IR promoted colon carcinogenesis. Cocarcinogenicity tests of neutral and acid steroids on mouse skin are underway.

Studies on the effect of diet and intestinal microflora in colon carcinogenesis show that sc dimethylhydrazine (DMH) led to colon cancer in 20 percent of germ-free but 93 percent of conventional rats. However, IR azoxymethane (AOM) induced more colon cancers in germ-free or mono-contaminated rats compared to conventional rats. The reciprocal experiment, IR DMH and sc AOM is underway. In a two-generation study of rats on high and low fat diets and chow, DMH gave

more colon cancer and more acid steroids in the stools on a high fat diet than on a low fat diet.

Significance to Biomedical Research and the Program of the Institute: Colon cancer constitutes one of the major types of cancer in the United States and in the Western World. The etiology of colon cancer, and factors and mechanisms related thereto are unknown. The current long-term effort is part of a key program to link animal experimentation and the study of human cancer causation. The project also serves as a flexible, skilled, well-equipped multidisciplinary central resource for the Colon Cancer Segment as regards wide-ranging laboratory investigations.

The information and new developments provided by this active research program are designed to yield ultimately an understanding of the contribution of exogenous and endogenous factors, such as diets, intestinal microflora, bile constituents, to the etiology of colon cancer in man. Evaluation of the main elements in this complex puzzle may eventuate in the formulation of a rational and sound set of preventive approaches.

Proposed Course: (1) to study carcinogenesis by dimethylhydrazine and 3,2'-dimethyl-4-aminobiphenyl in germ-free and microbially defined (gnotobiotic) rats, and to determine the effect of select bile acid, cholesterol, and tryptophan metabolites in these systems; (2) to study the fecal chemistry and microflora in animals on specific diets mimicking high and low risk populations in the presence and absence of colon carcinogens; (3) to investigate the effect of such diets on carcinogenesis in germ-free and conventional rats; (4) to continue studies on the in vitro metabolism by fecal microflora obtained from populations on various diets of endogenous metabolites such as bile acids, steroids, and other compounds with emphasis on the production of carcinogenic and/or cocarcinogenic materials; (5) to extend the study of the carcinogenicity or cocarcinogenicity on mouse skin and by intrarectal administration in rats of such endogenous metabolites; and (6) to act as a bioassay resource for the Colon Cancer Segment of the Carcinogenesis Programs.

Date Contract Initiated: June 30, 1971

Current Annual Level: $414,337

CALIFORNIA, UNIVERSITY OF (at Los Angeles) (NO1-CP-43290)

Title: Characterization of Mucosal-associated Microorganisms in the Human Colon and Relationship to Cancer and Pre-cancerous Conditions

Contractor's Project Director: Dr. Sherwood L. Gorbach

Project Officer (NCI): Dr. Sidney J. Silverman

Objectives: to define the structure and metabolic function of microorganisms intimately associated with the large bowel mucosa in man. The flora will be studied in healthy individuals and in patients with colon cancer. Mucosal biopsies will be examined by electron microscopy, and the microbial isolates

will be identified by biochemical procedures. The metabolic potential of the colonic flora is also being examined with regard to conversion of procarcinogens to carcinogens.

Major Findings: Intestinal biopsy specimens from 12 patients have been studied. The predominant microbial isolates in all colon biopsies were obligate anaerobes representing a wide variety of types. Species of Eubacterium, Lactobacillus, Clostridium, Bifidobacterium, Peptostreptococcus, Bacteroides and Fusobacterium were recovered from tissue homogenates. In general, the same organisms were recovered from the fecal flora, the surface of the biopsy and the tissue itself. At present it has not been possible to identify any specific bacteria, either anaerobic or facultative, that appeared to be uniquely associated with the mucosal surface. A possible exception was a specimen of colon cancer in which three different species of Clostridia were recovered in large numbers from deep in the tissue, whereas no Gram-positive rods were isolated from the mucosal surface.

Concurrent studies have been initiated to determine the metabolic activity of the flora and its relationship to colon cancer. An animal model has been employed to evaluate enzymological changes as a function of diet. The enzymes selected for study have been implicated in the activation of procarcinogens or the detoxification of carcinogens.

Preliminary experiments with rats fed either a cereal diet or a lean beef diet have shown significant changes in the specific activity of the fecal enzymes, β-glucuronidase, β-glucosidase and nitroreductase. The specific activity of β-glucuronidase and nitroreductase increased more than two-fold within 20 days after rats have been shifted from a cereal to a meat diet. In contrast, the β-glucosidase levels decrease fivefold within one week after changing to meat. The increase in glucuronidase and nitroreductase levels among rats maintained on a high meat diet is in agreement with previous studies that suggest these enzymes may activate procarcinogens by releasing a carcinogenic aglycone. Further studies are in progress to evaluate the effect of meat diets on the bacterial enzymes, N-dehydroxylase and azoreductase.

Significance to Biomedical Research and the Program of the Institute: Previous studies in rodents had identified a unique microbial flora associated with the colonic crypts. From results of preliminary studies, this mucosal-associated flora does not exist as a specific entity in man. Hence, the important metabolic events in colon cancer are related to the intraluminal flora.

Initial studies of microbial and mucosal enzymes have established that animals consuming a meat diet have an increased metabolic potential to activate carcinogens in their fecal flora. Investigations of several enzyme systems are planned to search for sentinel markers of high colon cancer risk.

Proposed Course: The major emphasis in future studies will be the metabolic activity of the colonic flora. The plan is to develop the enzyme assays in animals. As soon as the methods are established, studies in man will begin. Attention in the human investigations will be focused on high risk groups for colon cancer (ulcerative colitis, polyposis, strong family history and

previous resection of colonic cancer). The effect of diet on these fecal enzymes will also be examined with particular reference to Western diets and Chinese diets in the population of Boston's Chinatown.

Date Contract Initiated: May 16, 1974

Current Annual Level: $72,000

CALIFORNIA, UNIVERSITY OF (at Los Angeles) (NO1-CP-33380)

Title: Fecal Flora Studies

Contractor's Project Director: Dr. Sydney M. Finegold

Project Officer (NCI): Dr. Sidney Silverman

Objectives: To detect any differences in fecal microflora between subjects with a high risk (HR) of colon cancer and those with a low risk (LR). The first project compared two groups of 20 subjects each of Japanese ancestry, one of which consumed a primarily western diet (HR) and the other a primarily traditional diet (LR). The second project compared 25 subjects with a multiple adenomatous colonic polyps (HR) with 25 matched controls with negative colonoscopy (LR).

A third study, in progress, is comparing two Seventh Day Adventist populations, one consisting of strict vegetarians and the other of moderate meat eaters (25 in each group). In addition, a larger number of subjects is being studied only for the presence of the Clostridium paraputrificum group of organisms (this group is thought by British workers to be a key group in terms of carcinogen production). Included are 35 people in each of the above two Seventh Day Adventist groups plus 35 non-Adventists on a conventional American diet.

Better selective and non-selective media are being developed and methods for simplifying intestinal bacteriologic techniques are being studied.

Major Findings: In the Japanese-Western diet study, the HR group had significantly lower counts of S. faecalis var. faecalis, of certain miscellaneous facultative or aerobic bacteria, of two species of Eubacterium and of certain unclassifiable peptostreptococci. Certain Bifidobacterium infantis subspecies and Peptococcus magnus were present only in the HR group.

The polyp patients had significantly higher counts of B. adolescentis, E. rectale and several anaerobic cocci (Veillonella, Ps. productus, Pc. saccharolyticus and Sarcina). The controls (LR) had higher counts of B. infantis and Bacteroides putredinis and had E. tenue and E. combesii present more frequently.

In neither project were there significant differences between HR and LR groups with regard to Clostridium paraputrificum or related organisms.

The results are being studied presently for significant differences re: combinations of organisms. Additional studies are indicated; the data may show more consistent trends when a larger number of subjects has been studied.

In the Seventh Day Adventist study, 21 major specimens have been studied to date, along with 38 specimens for the C. paraputrificum group. Bacteriologic studies are not yet completed in many cases and, of course, the code will not be broken until the study is completed, so results cannot yet be analyzed.

The studies continue to provide valuable information on the normal human fecal flora and on optimum media and techniques for defining this flora accurately and expeditiously. A major advance has been the development of a cycloserine mannose selective medium which facilitates rapid recovery and identification of Clostridium butyricum and Clostridium paraputrificum. With this medium it is often possible to recover these organisms when they cannot be detected on the usual media used. Rifampin blood agar has been similarly useful for recovery of other members of the C. paraputrificum group.

Significance to Biomedical Research and the Program of the Institute: To date, a great deal has been learned of the nature of the normal human fecal flora and of optimum ways of defining it. This information will be useful in many ways other than its possible relationship to colon (and breast) cancer.

A number of striking differences in fecal flora composition between high and low risk groups have been noted. Statistical analysis of the data from the first 90 specimens (Japanese-Western diet study and polyp-control study) is still in progress. It is clear, however, that differences which might be important are restricted to about 75 of the approximately 300 different species recoverable from fecal specimens. Further studies could be concentrated on this smaller group of organisms. Among these 75 may be certain key organisms which predispose to, or protect against, cancer. To date, data do not support the contention of a British group that C. paraputrificum and related organisms may play a key role in predisposing to bowel cancer. It is anticipated that the further statistical analysis currently in progress plus the data from the Adventist study will further narrow down the field of organisms which may be of significance.

Proposed Course: The next phase will be the study of patients with early carcinoma of the bowel (without obstruction or bleeding) in comparison with matched controls, and the continuation of efforts to improve and simplify bacteriologic techniques. Future protocols will concentrate on a limited number of organisms of potential interest, as revealed by data analysis, so as to be able to study larger numbers of people.

Date Contract Initiated: June 1, 1971

Current Annual Level: $163,351

GEORGIA, MEDICAL COLLEGE OF (NO1-CP-43282)

Title: Epidemiologic Study of Colon Cancer Among Blacks

Contractor's Project Director: Dr. Warren H. Gullen

Project Officer (NCI): Dr. Sidney J. Silverman

Objective: To determine the relationship of diet (especially animal protein, fat, and refined carbohydrates) to the occurrence of cancer of the colon.

Major Findings: The design phase has been completed. All instruments needed for field work have been completed. Nine hospitals in the Atlanta area have enrolled and negotiations are underway with an Atlanta area health department for performance of field work which will start in the near future.

Significance to Biomedical Research and the Program of the Institute: Successive cancer surveys by NCI have shown that the incidence rate for colon cancer among Southerners has been and remains lower than the rate for Northerners. Indeed, between the last two national NCI cancer surveys, the rate for Northern blacks has risen to equal that among Northern whites; while the rate for Southern blacks remains lower than that for Southern whites which in turn is lower than that for Northerners. This observation, along with the results of studies conducted by NCI and others, tends to implicate diet as an etiological factor. Attempts to confirm the etiological role of diet in studies of relatively high-colon-cancer-rate populations have, to date, been unsuccessful. Therefore, this study is being executed among a population with a relatively low colon-cancer incidence to derive more definitive data bearing on the hypothesis.

Proposed Course: To conduct a retrospective case-control study of colon cancer in nine Atlanta area hospitals, with investigation of differences in dietary history between persons with and without colon cancer.

Date Contract Initiated: June 30, 1972

Current Annual Level: $63,315

KAISER FOUNDATION RESEARCH INSTITUTE (NO1-CP-33215)

Title: Epidemiological Study of Colon Cancer in Blacks

Contractor's Project Directors: Dr. Gary D. Friedman
Dr. Loring G. Dales

Project Officer (NCI): Dr. Sidney J. Silverman

Objectives: To explore potential associations between bowel cancer and intake of meats (particularly beef), saturated fats, foods treated with nitrite preservatives, and staple foods low in unrefined carbohydrate or roughage.

Major Findings: This is a case-control study of diet and bowel cancer among San Francisco Bay Area Blacks. Interviews are being conducted on 150-200 bowel cancer cases, together with three age, race, and sex-matched controls for each. The interview concerns dietary practices before illness onset, but also explores occupation, education, residence history, smoking habits, obesity and other bowel disorders.

In the first year of operation 42 cases and 78 controls were interviewed. Initially, about half of the potential cases identified could not be interviewed due to death before interview, incapacitation, failure to meet dietary practice or diagnostic criteria, inability to be located, or physician or patient refusal. Improved methods of identifying and finding cases appear to be reducing this loss of cases.

Interviewed cases ranged in age form 29 to 83 years (mean, 59 years), and 57 percent were men. Subjective interviewer ratings of subjects' reliability were high at all ages. A preliminary analysis for case-control differences in dietary practices is in progress, and thus far no significant differences have emerged.

Significance to Biomedical Research and the Program of the Institute: A positive association between meat (especially beef) consumption has been seen in case-control studies of Japanese-Hawaiians and Seventh Day Adventists, though previous studies performed on Caucasian American subjects had failed to discern this relationship.

It is of importance to determine if this dietary lead, as well as the important Burkett hypothesis concerning unrefined carbohydrates, can be corroborated in other populations.

Proposed Course: The analysis of the first year's data will be completed; data collection on additional cases and controls will continue, so that definitive analysis can be conducted, based on a more substantial number of subjects.

Date Contract Initiated: December 1, 1972

Current Annual Level: $30,320

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CP-43366)

Title: Analysis of Regulatory Control of Cell Proliferation in Normal, Premalignant, and Malignant Colonic Tissue in Familial Polyposis

Contractor's Project Director: Dr. Martin Lipkin

Project Officer (NCI): Dr. Ann E. Kaplan

Objectives: To define the early changes initiating carcinogenesis, and molecular events responsible for the increased susceptibility to neoplastic transformation observed in this inherited disease of man that leads to colon

cancer; and to develop laboratory screening tests for early detection of these changes and a resource of human cells with varying degrees of transformation susceptibility for use in the Carcinogenesis Program to identify carcinogens and their mode of action in man.

Major Findings: (1) Abnormal Proliferation of Colonic Epithelial Cells in Familial Polyposis-- In eight index cases of familial polyposis and symptom-free relatives, biopsies of colonic mucosa and microautoradiography showed incorporation of ^{3}HTdR into surface epithelial cells in flat colonic mucosa and in polyps. Findings indicated the development of cells with persistent DNA synthesis at the surface and along the upper portion of colonic crypts. The defect was focal and widespread in the colon.

(2) Early Detection of Increased Proliferative Activity in Colonic Epithelial Cells in Familial Polyposis--Additional experiments indicated that persistent DNA synthesis could be detected in surface epithelial cells washed out of the colon by pulsatile saline lavage. Initial experiments in mice given the chemical carcinogen 1,2-dimethylhydrazine (DMH) and patients with familial polyposis suggested the procedure could be used to detect abnormal thymidine labeling of surface epithelial cells before the development of adenomas.

(3) Effect of a Naturally Occurring Low Molecular Weight Compound on DNA Repair--Evidence was provided that pyridoxal 5'-phosphate could influence the modulation of DNA repair. Initial studies were carried out in a cell-free system, and subsequent experiments with a colon carcinoma cell line suggested that modification of the F_1 histone fraction by the ligand was involved.

In addition to experiments on intestinal cells, parallel experiments are being carried out to study transformation of cutaneous fibroblasts removed from individuals with familial polyposis. Cells from proband and other polyposis family members and controls are being studied. Experiments underway include definition of growth characteristics and factors associated with transcription.

Significance to Biomedical Research and the Program of the Institute: There is every reason to believe that the adenoma-cancer transition is the same in multiple polyposis as it is in colon cancer generally. It seems quite possible that the earlier stages of tumorigenesis might also be the same. If so, the polyposis syndrome, caused by a single gene mutation, would be a most productive place to look for the earliest possible marks of carcinogenesis. Also, by identifying the underlying molecular alteration, one could be a great deal closer to determining what type of environmental factor is involved. It is submitted that genetic defects of this type have been the most fruitful source of our understanding of the molecular pathology underlying human disease and that this study of colon cancer should take advantage of the existence of this appropriate experiment in nature.

Proposed Course: Studies will be continued to evaluate the biochemical changes associated with phenotypic expression of neoplastic transformation, both in colonic epithelial cells and fibroblast cells of patients with familial polyposis. Cell growth in immunodeficient mice and selective media is being analyzed. The transition of cells through specific phases of the proliferative cell cycle wil be studied to differentiate normal from transformed cells.

Measurements will be carried out to identify changes in gene expression and transcription in cells of man in different stages of transformation. Laboratory screening tests are being developed to detect and screen increased susceptibility to transformation in individuals with this genetic disease, and a resource of human cell lines is being established to aid the identification of carcinogens and their mode of action.

Date Contract Initiated: October 15, 1971

Current Annual Level: $94,857

MISSOURI, UNIVERSITY OF (NO1-CP-33335)

Title: Effect of a High-Meat Diet on the Bacterial Flora and Chemical Components of Feces

Contractor's Project Director: Dr. David J. Hentges

Project Officer (NCI): Dr. Sidney J. Silverman

Objectives: To determine if a high-meat diet alters the bowel flora and the chemical composition of feces of humans.

Major Findings: In examining the influence of diet on the bacteriological composition of the intestinal flora of humans, there are great variations from individual to individual in the populations of most genera of bacteria. Despite individual variations, the populations of any given genus varied little with dietary change when results from all ten volunteers in the study were averaged. The exception occurred with the genus Bacillus. There was a significant increase in its population in the feces when volunteers consumed a high-meat diet. A gradual increase in the total anaerobe population during the diet periods may be related to stress factors associated with consumption of the same diet over each four-week period of time. Analysis of data on the various chemical determination are incomplete so that conclusions cannot be drawn at this time.

Significance to Biomedical Research and the Program of the Institute: Epidemiological studies indicate that a relationship exists between the incidence of colon cancer and the quantity of meat consumed by a population. A systematic study of the effects of a high-meat diet on the composition of the intestinal contents of humans had never before been undertaken. Results will provide information on changes that occur in the fecal flora and in the concentrations of potentially carcinogenic compounds present when individuals are placed on a high-meat diet. This information is essential to an understanding of the role of diet in the etiology of colon cancer.

Proposed Course: It is expected that data analysis of results and preparation of several manuscripts will be completed by June 1975.

Date Contract Initiated: June 5, 1973

Current Annual Level: $119,640

NCI, FREDERICK CANCER RESEARCH CENTER (LITTON BIONETICS INC.) (NO1-CP-25423)

Project 7

Title: Laboratory Studies of Etiology of Cancer of the Digestive System

Contractor's Project Manager: Dr. Milton Slein

Project Monitor (NCI): Dr. Sidney Silverman

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

OXFORD, UNIVERSITY OF (NO1-CP-23215)

Title: Study of the Relationship in Individuals between Colon Cancer and Epidemiologically-Linked Diseases

Contractor's Project Director: Dr. John Baldwin

Profect Officer (NCI): Dr. Sidney Silverman

Objectives: To investigate hypotheses that certain diseases (such as coronary heart disease) are caused by the same factors as specified bowel tumors, and will, therefore, be linked in individuals in a general population more frequently than would be expected by chance.

Major Findings: Analysis of the linkage of colon cancer with other diseases in England, and the geographic distribution of colon cancer in England. In the former analysis, the highest correlation was with atherosclerosis.

Significance to Biomedical Research and the Program of the Institute: The association of colon cancer and other diseases in populations has been the subject of much discussion. Determination of the links in individuals would provide invaluable clues to etiology and, as well, provide markers of high risk for detection programs. While this kind of information is sought from U.S. sources, all are small and/or highly selective populations, and the data are not readily accessible to say the least. Major segments of later parts of the program will be based on these epidemiological findings.

Proposed Course: Completion of report on the geographic distribution of colon cancer cases in England. Geographic distribution of colon cancer in England will be sought through existing computerized information.

Date Contract Initiated: April 9, 1972

Current Annual Level: $12,000

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY (NO1-CP-33334)

Title: Comparative Fecal Flora Studies

Contractor's Project Directors: Dr. W. E. C. Moore
Dr. L. V. Holdeman
Dr. T. D. Wilkins

Project Officer (NCI): Dr. Sidney Silverman

Objectives: To determine the bacterial fecal and colon flora of persons of several populations that are at different statistical risks of colon cancer, and to study differences in metabolism of neutral steroids among persons who have different statistical risks of colon cancer.

Major Findings: Bacteriological studies have been completed on the fecal flora of 20 Japanese-Hawaiians, 20 polyp patients, 20 native Japanese, 20 native South Africans and five persons on each of three extreme diets (rice, leafy vegetables, then beef). The extreme dietary changes did not show the expected major change in the fecal flora. The fecal bacteria present on all diets had a low level of proteolytic activity and many required complex carbohydrates for growth. This suggests that a major nutrient may be the intestinal mucin. Statistical comparisons of the flora of the various populations is now in progress. Bacteriological studies have been initiated on the flora of eight different areas of the gastro-intestinal tract. Bacterial flora of three tracts have been completed.

Fecal neutral steroids of 100 normal North Americans between 5 and 25 years of age have been analyzed by gas chromatography. No differences were found in the percent cholesterol converted to coprostanol and coprostanone between males and females or different age groups. The fecal neutral steroids of 38 South African natives were compared to the North American group. Cholesterol was more extensively degraded in the Africans. This result is contradictory to the theory that more extensive cholesterol conversion is associated with high risk of colon cancer.

Significance to Biomedical Research and the Program of the Institute: Correlation of types of flora with populations who are at different risk, and with nondegradation of neutral steroids may help elucidate mechanisms of intestinal carcinogen production.

Proposed Course: Comprehensive analysis of the important previously undescribed major bacteria species the flora are planned and statistical analyses of the present data base (approximately 10,000 characterized bacterial isolates) according to species and metabolic group. Investigations of the bacteria of the flora that are important in neutral steroid metabolism are also planned.

Date Contract Initiated: June 30, 1971

Current Annual Level: $182,686

SUMMARY REPORT

INFORMATION AND RESOURCES SEGMENT

July 1, 1974 through June 30, 1975

The Information and Resources Segment (IRS) has three major areas of responsibility within the Carcinogenesis contract program. These are the broad general categories of animal, chemical, and information resources. Within these categories, the Segment responds to the need to provide support to other Segments with resource contracts; it also provides for the anticipation, planning and provision for services on resource needs of the total Carcinogenesis Program.

It is projected that the Segment's activities at the end of this fiscal year will include 24 contracts and a total budget of approximately $2 million. Slightly more than half of these funds will be expended in the area of information services; the greater part of the remainder will be devoted to chemistry resources, with a somewhat lower level of activity in the animal resources area.

Chemical Resource Activities:

For the past three years a carcinogen reference bank has been in existence. The contractor is acquiring, purifying and characterizing specified compounds and repackaging them in smaller quantities for storage and distribtuion to investigators in the cancer research community. Over 70 such materials are currently available and new ones are being added on a continuing basis. The reference bank is temporarily located at the NCI Frederick Cancer Research Center (Litton Bionetics, Inc.) (NO1-CO-25423) while its permanent location is being determined.

Due to the Occupational Safety and Health Administration's (OSHA) regulations for handling carcinogens, many commercial suppliers have discontinued the manufacturing of carcinogenic compounds. Thus the Carcinogenesis Program has become the only source for many of these chemicals. Because of increased demand, the preparation program of the reference compound bank will be expanded in the coming year, and an RFP has been issued seeking organizations that can prepare or purify as well as analyze hazardous chemicals in facilities which will comply with the OSHA regulations. An award is expected before the end of this fiscal year.

A new synthesis program for isotopically labeled N-nitroso compounds has been initiated. Of the contractors participating in this program, two (Stanford Research Institute [NO1-CP-55672] and Research Triangle Institute [NO1-CP-55671]) have basic ordering agreements for the preparation of needed compounds. The third (University of New Hampshire [NO1-CP-55675]) will be developing improved methodology for the preparation of heterocyclic N-nitroso compounds.

The Segment intended to make the compounds prepared by the N-nitroso compound synthesis program and the compounds in the reference bank available to the scientific community. The question of whether or not this distribution was legally permissible was directed to the Office of the General Counsel, DHEW. The Public Health Services Act and the National Cancer Act permit distribution of biological materials and therapeutic agents, but neither of the legislative documents mention chemicals. Therefore, the Office of the General Counsel replied that distribution be limited only to contractors of the National Cancer Institute. The Segment would like to see this ruling modified or the legislation amended to permit broader distribution of chemicals.

The IIT Research Institute (NO1-CP-33296) provides highly purified and characterized powders and suspensions for use by the Lung Cancer Program in intratracheal instillation studies. The suspensions are composed of carcinogenic materials (or their non-carcinogenic analogs) in aqueous systems, are of high purity, and have known size distributions. The powders are prepared from carbon and a variety of metal oxides and have defined size distribution, shape, surface area, and porosity. The powders are provided to investigators either alone or after coating with benzo[a]pyrene.

The collaborative program with Ash Stevens, Inc. (NO1-CP-23293) continues to provide interested investigators with purine and pyrimidine nucleotides specifically tailored to function as selective irreversible inhibitors of nuclear RNAse. This aspect of the program is attempting to develop a selective drug probe enabling investigators to control the destruction of messenger RNA in the cell nucleus. In addition, a number of these products have been shown to function as inhibitors of the reverse transcriptase of oncogenic viruses and leukemia cells.

Five contracts, forming a cooperative project to develop new and improved methodology for the determination of N-nitroso compounds in the environment, are completing their first year of operation with promising results. This program is a joint effort with the Food and Drug Administration. At the end of the first year, this area will be transferred administratively to the Carcinogen Metabolism and Toxicology Segment from which the funding had originated.

A feasibility study to identify human populations at risk due to exposure to N-nitroso compounds being conducted by the Mt. Sinai School of Medicine (NO1-CP-43255) is near completion. The final report is expected before the end of this fiscal year.

Finally, a task has been established at the NCI's Frederick Cancer Research Center (NO1-CO-25423) to establish methodology and guidelines for the safe-handling of carcinogenic chemicals including safe methods of disposal.

Animal Resources:

The Segment continues to support the development of the wild European hamster (Cricetus cricetus L.) as a versatile animal model at the Medizinische Hochschule Hanover (NO1-CP-12148). The domestication of these animals

continues to be successful and the colony now has achieved F_7 generation. The contractors report increasing interest in the animal both in Europe and the United States. When possible, breeding animals will be supplied with the understanding that the NCI requests will always have the highest priority. A black feral animal has been captured and attempts will be made to develop a line from it. One shipment of ten males and ten females has been received and is now quartered at the NIH Animal Center at Poolesville. Arrangements are being made to import another similarly sized shipment. It is anticipated that the animals at Poolesville will begin to breed this spring and the colony should rapidly enlarge.

The investigators at Hannover have written an excellent anatomical atlas for this species. The Segment proposes to invest sufficient funds to edit it and make it more suitable from the position of American veterinary taxonomy. Alternate pathways for publishing this book will then be considered.

A contract has been established at the Charles River Breeding Laboratories (NO1-CP-55651) for the production of $B_6C_3F_1$ mice and F344 rats. The production activity will be supported by a pedigreed nucleus maintaining both germ-free and associated flora. The desired monthly production level, to be reached approximately one and one-half years after award, is 1,000 animals of each species.

An RFP for the production of two strains of aged male rats required for the prostate cancer research program has been advertised. Proposals are now being evaluated with a contemplated award date of June 1, 1975. The RFP addressed the need for a colony numbering 2,000 males of each strain. Individual strain awards could be made if it is found desirable.

The Institute of Laboratory Animal Resources (ILAR) of the National Academy of Sciences/National Research Council (NO1-CP-45617) is completing a document titled "Standards for Long-Term Rodent Holding" as a follow-up to the workshop held last year. At the same time, also by the ad hoc committee approach, ILAR is assembling a document on the athymic (nude) mouse that will encompass the state of the art for this unique animal model with respect to its genetics, husbandry and uses in biomedical research. It is contemplated that both of these publications will be prepared before the end of FY 1975.

Information Resource Activities:

Activities of the Segment in this area are designed to meet a variety of information needs generated by the scientist or administrator working in carcinogenesis research.

A continuing need exists to make more easily available, on a timely basis, the relevant information being generated in those disciplines which have an impact on the Carcinogenesis Program. This requirement is being met in part by activities such as the secondary journal, Carcinogenesis Abstracts, produced under contract with the Franklin Institute Research Laboratories (NO1-CP-43293). In order to increase the utility and dissemination of this

information product, Volume 12 contains, besides a subject and author index, a Chemical Abstracts Service Registry Number and a Wiswesser Line Notation index. Dissemination is to be enhanced by the inclusion of the abstracts in a subset of Toxline (the on-line bibliographic data base of the National Library of Medicine) called Cancerline.

Of high priority for program use is information which explores the carcinogenic potential of chemicals or classes of chemicals in animal test systems. To ensure maximum usefulness of the efforts of the scientific community, this data and information has been gathered and extracted from the scientific literature by a contract with Tracor Jitco, Inc. (NO1-CP-33402). The volume covering the 1972-1973 literature of the publication series entitled Survey of Compounds Which Have Been Tested for Carcinogenic Activity (PHS 149) is now in press. To make the data and information of the various volumes more available, master cumulative indexes of the entire series were prepared for inclusion in the 1970-1971 volume. These indexes, which include chemical nomenclature, Wiswesser Line Notation, molecular formula, Chemical Abstracts Service Registry Number, species of animal used, vehicle, site of administration and site of induced tumor, were also produced in machine readable form. These indexes will also be available in the on-line toxicology data base, Toxline.

The growing concern with our abilities to predict the carcinogenic potential of molecules based on reports of other biologic effects has caused the Segment to furnish partial support for the Environmental Mutagen Information Center (EMIC) located at the ERDA's Oak Ridge National Laboratory (YO1-CP-20203). This program functions in the mutagenesis area in a fashion similar to literature-related activities in the Carcinogenesis Program. Elements of chemical-mutagenesis information are being extracted into the EMIC data base in a manner which permits easy linkage to the index portions of PHS 149.

In continuing collaboration with the International Agency for Research on Cancer (IARC) (NO1-CP-45608), Volumes 5, 6, and 7 of the IARC monograph series entitled Evaluation of Carcinogenic Risk of Chemicals to Man have been published and are subtitled "Some Organochlorine Pesticides" (Volume 5), "Sex Hormones" (Volume 6), and "Some Anti-Thyroid and Related Substances, Nitrofurans and Industrial Chemicals" (Volume 7). Volume 8 is now in press and is concerned with the azo dyes. Also produced in collaboration with IARC were Issues 3 and 4 of the "Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity." This Bulletin lists chemicals under test throughout the world, including information on the status of the study. The Bulletin should be useful in eliminating duplication of effort in carcinogenicity testing and thereby making most effective use of limited resources.

An attempt to develop mechanisms which will assist scientists in making decisions as to which chemicals should receive priority in testing or investigational programs, collaboration is underway with the Stanford Research Institute (NO1-CP-33285) to develop a program having as its objective the collection, analysis, and systematization of information on

the use, production, and extent of human exposure for chemicals which have significant contact with man. The categories of chemicals which have been analyzed for inclusion in the data base to date include intentional food additives, pesticide residues in food, proprietary drugs, prescription drugs, cosmetics, air pollutants, soaps and detergents and trade sales paints. Under evaluation for inclusion are agricultural chemicals, sealants and caulkings, and industrial chemicals. This data base and its supporting systems are transferable to our in-house computer complex.

The first year of a new approach to handling information needs which require quick response has been completed. A basic ordering agreement has been established with the Franklin Institute Research Laboratories (NO1-CP-55634). In the first year, 16 tasks were issued against the basic ordering agreement. Several tasks are now in process of being defined.

CONTRACT NARRATIVES

INFORMATION AND RESOURCES SEGMENT

July 1, 1974 through June 30, 1975

ASH STEVENS, INC. (NO1-CP-23293)

Title: Synthesis of Purine and Pyrimidine Nucleotides

Contractor's Project Director: Dr. Arthur B. Ash

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The broad aim of this contract is to design and make available a new type of pharmacological agent or agent which will be useful in investigating the manner in which chemical and viral carcinogens affect the metabolism of newly-synthesized and messenger RNA in the cell nucleus, as well as for the investigation of a variety of other problems in chemical and viral carcinogenesis. The specific aim of the contract is to synthesize suitably modified nucleotides which will irreversibly inactivate the nuclear enzyme which destroys newly-synthesized and messenger RNA in the nucleus itself; the agent must be selective and biologically active. An additional contract aim is the assay of synthesized compounds for inhibition of the RNA-dependent-DNA polymerase (reverse transcriptase) of oncogenic viruses and leukemic cells. This aspect of the project has been implemented and has yielded important results. A number of compounds were screened for polymerase activity and some preliminary data have been obtained. Sufficient KB tumor cell data have been obtained to define closely cytotoxicity structure-activity relationships.

Major Findings: Chemical synthesis in the past year has emphasized two phases of work. The work in oligodeoxythymidylates has been carried successfully to tetramers, hexamers and, recently, dodecamers. In each series, two optimal reactive groups have been placed in the 3'-, 5'- and 3', 5'-terminal positions. The second phase of work, receiving heavy emphasis in the past three months, is the enzymatic preparation of 2'-O-methyl- and 2'-amino-2'-deoxyuridine nucleotide polymers and copolymers suitable for chemical conversion to inhibitors. Overall, some 40 inhibitors were distributed to various investigators. A major contract milestone was met by significant inhibition of the exoribonuclease concentrations as low as 10^{-10} M(0.0002 µg/ml) with a specificity of several orders of magnitude relative to vital cellular enzymes. Additional work with reverse transcriptase has confirmed the original findings of 50% inhibition at 10^{-6} M. Tests with the first dodecathymidylate (an oligo dT inhibitor), which is capable of hybridization by base-pair binding with poly A, are awaited with great interest. An initial lead against herpes simplex virus failed in a second test, but a broad spectrum of testing is planned. Limited KB cell cytotoxicity gives a clear delineation of structure dependency upon the reactive group.

Significance to Biomedical Research and the Program of the Institute: Within the past few years, there has accumulated an important body of data which show that, in many different vertebrate cell types, most of the RNA that is made in the cell nucleus is destroyed in the nucleus itself very shortly after synthesis. Published data indicate that as much as 90% of the newly-synthesized RNA is destroyed in the nucleus and that its half-life may be only a few minutes. This entire field is still virtually untouched with respect to the problem of carcinogenesis. It is well established that chemical carcinogens have immediate and profound effects on the metabolism of RNA in the cell nucleus. The development of a selective drug probe, which would enable the investigator to stop the destruction of messenger RNA in the cell nucleus (without interfering with its synthesis), would open up a wide variety of experimental work in the field of chemical or viral carcinogenesis. Moreover, the synthesis and testing of potential inhibitors of reverse transcriptase, as well as other important cellular enzymes, a field in which this contract has already yielded successful results, are areas of high priority and relevance in other aspects of the NCI total program.

Proposed Course: To continue to develop new synthetic polynucleotide analogs by utilizing enzymatic synthesis to obtain longer chain length oligomers as carriers of the reactive (irreversible-inhibiting) groups either at the terminal or internucleotide positions. In addition, direct chemical introduction of reactive groups into suitable high molecular-weight synthetic polymers, which are the natural substrates of a given target enzyme, will be studied. The compounds will be evaluated against the nuclear ribonuclease and other important target enzymes which may play a role in carcinogenesis with implementation in whole cell bioassays.

Date Contract Initiated: June 21, 1972

Current Annual Level: $140,090

CHARLES RIVER BREEDING LABORATORIES (NO1-CP-55651)

Title: Establisment of a Gnotobiotic Originated Rodent Production Colony

Contractor's Project Director: Dr. George J. Pucak

Project Officer (NCI): Dr. Thomas Cameron

Objectives: To develop a rodent production colony conducted under the best concepts of what is commonly considered a "barrier" system that will in the shortest possible time attain an issuable weanling level of 1,000 Fischer 344 rats and 1,000 B_6 C_3 F_1 hybrid mice (C_{57} Blk X C_3H), of equal sex distribution on a monthly basis. The nucleus colony will be self-sustaining and on a yearly basis, germfree pedigreed litters will be furnished to the contractor from the genetic unit of NIH.

Major Findings: Pedigreed litters of C_3H/HeN, C_{57} Blk/6N and F-344/N mice and rats have been received from the genetics unit inhouse at NIH. Original animals were put into nucleus colonies and will, as they reproduce, be divided

into expansion colonies and finally production colonies. As expected, the C_{57} Blk/6's have created the most difficulty from a production aspect and since we have only recently received the F-344/N nucleus stock, no comment can be made. No problems are anticipated with these animals and availability of animals should coincide with or before contract predictions.

Significance to Biomedical Research and Program of the Institute: The significance is that this contract will provide a constant and dependable source of animals to various NCI contractors, so they will then be able to schedule and continue their programs which utilize the strains being produced. It will also insure that those investigators performing related experiments will be using a standard biological model.

Proposed Course: To continue to breed nucleus stocks which will be put into expansion and ultimately into production colonies.

Date Contract Initiated: October 1, 1974

Current Annual Level: $66,588

ERDA-NCI INTERAGENCY AGREEMENT (OAK RIDGE NATIONAL LABORATORY) (YO1-CP-20203)

Title: Environmental Mutagen Information Center (EMIC)

Contractor's Project Director: Dr. James S. Wassom

Project Officer (NCI): Dr. Sidney Siegel

Objectives: The mission of the Environmental Mutagen Information Center (EMIC) is to collect, organize and disseminate chemical mutagenesis information. Information contained in this unique file may either report on the testing of chemicals in one of the many available mutagenic assay systems or contain pertinent data which can be used in understanding the known or suspected mutagenic activity of environmental chemical agents. The information collected for this data base is to be processed and disseminated to the scientific community.

Major Findings: Approximately 1,500 references were added to the EMIC data base during FY '75. This figure brings the total number of references on file to approximately 15,500. All of these citations have been indexed with Agent/Organism keywords and Chemical Abstracts Registry Numbers. Correlations with information available in PHS 149 were made with compounds in the EMIC Registry. Additionally, a literature review was made on microbial assay systems used in the evaluation of potential carcinogenic chemicals for mutagenic activity. The assay system which appears to be the most sensitive is one which combines treatment of the test chemical with a liver homogenate or microsome fraction prior to its evaluation for the induction of reverse mutations in highly sensitive and genetically characterized Salmonella typhimurium strains.

Significance to Biomedical Research and the Program of the Institute: Mutagenesis and carcinogenesis are complex processes which may be related in their mechanism(s) of action. In the last few years, as more knowledge has been accumulated about the structure and metabolic fate of chemicals with respect to their biological activity, a general qualitative correlation between mutagenicity and carcinogenicity has been suggested. Additionally, mutagenicity test systems are also being investigated as potential prescreens to detect potential carcinogenic activity.

EMIC is correlating the availability of carcinogenesis information with the 5,500 compounds presently in its data base. This correlation, along with EMIC's indexed literature collection, makes it possible for the program of the National Cancer Institute to respond to its own and other queries for experimental data. This activity will assist the NCI and its contractors in carrying out the responsibilities stated in the National Cancer Act of 1971 which is to "collect, analyze, and disseminate all data useful in the prevention, diagnosis, and treatment of cancer including the establishment of an international cancer research data bank to collect, catalogue, store, and disseminate insofar as feasible the results of cancer research undertaken in any country."

Proposed Course: As the result of increasing interest in the correlation between mutagenesis and carcinogenesis, it is highly desirable that such a data base concerned with biological response to exogenous chemicals be maintained on a continuing basis.

The scientific literature shall be continuously monitored and all papers reporting the mutagenic effects of compounds in the human environment will be collected and added to the data base. Efforts will be continued to find more expedient methods of making this data file more readily available to interested individuals. Publication of selected data in tabular form will be continued. Reviews and special literature compilations will be done on areas of concern. Inputting of literature published before 1969 with respect to agents tested will be done to the extent that time permits.

Date Contract Initiated: June 30, 1972

Current Annual Level: $75,000

EXPERIMENTAL PATHOLOGY LABORATORIES, INC. (NO1-CP-43300)

Title: Animal Pathology Support

Contractor's Project Director: Dr. William M. Busey

Project Officer (NCI): Dr. Robert A. Squire

Objectives: (1) To provide consultation services to the Carcinogenesis Program, (2) to perform the histopathological evaluation of animal tissues from

intramural and extramural programs within the Carcinogenesis Program,and (3) to provide training in histologic techniques for contractor personnel in the Carcinogenesis Program.

Major Findings: During the past year a histopathological evaluation of rats and mice receiving aldrin, chlordane and heptachlor was conducted. Preliminary evaluation of these studies indicates that aldrin, chlordane and heptachlor are carcinogenic in the mouse, resulting in the development of hepatocellular carcinomas.

The trisodium salt of nitrilotriacetic acid (NTA) was also found to be carcinogenic in the rat at 20,000 ppm of the diet. Primary malignant neoplasms of the urinary system were found in approximately 40% of the females and 50% of the males receiving 20,000 ppm of NTA for 78 weeks.

Significance to Biomedical Research and the Program of the Institute: The pathology services provided by Experimental Pathology Laboratories allow for the timely completion of the histopathologic evaluation of sensitive compounds under investigation in the Carcinogenesis Bioassay Program. The pathologists at Experimental Pathology Laboratories are available for consultation and for processing animal tissues for histologic examination. Similarly, the histology laboratory at Experimental Pathology Laboratories is available for the training of intramural and extramural histology technicians.

Proposed Course: To continue this pathology support service to the Carcinogenesis Program for as long as it is required.

Date Contract Initiated: March 20, 1974

Current Annual Level: $90,996

FRANKLIN INSTITUTE RESEARCH LABORATORIES (NO1-CP-55634)

Title: Information Resource

Contractor's Project Director: Dr. Paul N. Craig

Project Officer (NCI): Dr. Sidney Siegel

Objectives: This project is a resource providing the capability of rapid responses to information needs to the Carcinogenesis Program.

Major Findings: By the use of a basic ordering agreement, it has been possible to structure information tasks (which are defined by the requestor, the project officer and the contractor's project director) by screening the scientific literature for relevant data and information on selected narrow areas of research and by gathering, collating and evaluating data and information on areas of research which have short- and long-range implications on decisions making for the scientist and scientist administrator.

Significance to Biomedical Research and the Program of the Institute: All aspects of scientific research have information needs ranging from searching the current literature to preparing critical reviews which are necessary for the optimal development of a research program. There has been a phenomenal increase of published literature in the past several years including a proliferation of journals. Since carcinogenesis research encompasses a number of scientific areas, much time and effort can be expended by an individual investigator in pursuing information needs, some of which can be readily handled and others of which the resources are not available but are necessary for the proper development of research.

This project provides the resources for satisfying information needs of investigators and science administrators in the Carcinogenesis Program in a timely and effective manner.

Proposed Course: The contractor will continue to respond to the information needs of the Program.

Date Contract Initiated: October 25, 1973

Current Annual Level: Since this is a Basic Ordering Agreement, the level of expenditure is assigned to each task as it is developed.

FRANKLIN INSTITUTE RESEARCH LABORATORIES (NO1-CP-43293)

Title: Preparation of Carcinogenesis Abstracts (Volumes 12, 13 and 14)

Contractor's Project Director: Dr. Bruce H. Kleinstein

Project Officer (NCI): Dr. Sidney Siegel

Objectives: To provide an up-to-date abstract service for carcinogenesis researchers throughout the world in a format designed to provide under one cover scientific work in the various disciplines which relate to cancer etiology.

Major Findings: This contractor has performed well and delivered the required product on time.

Significance to Biomedical Research and the Program of the Institute: The multidisciplinary approach used in the study of carcinogenesis needs an information exchange medium which gathers the relevant articles under one cover and once done allows for intellectual cross fertilization among the disciplines to produce a more rapid evaluation and use of information in the study of carcinogenesis.

Proposed Course: To continue the publication for an indefinite period of time and to include all relevant material in each issue instead of a set number of abstracts and/or citations.

Date Contract Initiated: February 1, 1973

Current Annual Level: $345,019

IIT RESEARCH INSTITUTE (NO1-CP-33296)

Title: Production and Characterization of Particulate Materials for Respiratory Studies

Contractor's Project Director: Ms. Jean Graf

Project Officers (NCI): Dr. John Cooper II
Dr. David Kaufman

Objectives: (1) To prepare and characterize any powder fractions needed for lung retention studies, (2) to prepare and characterize powder fractions coated with any polynuclear hydrocarbon carcinogen of interest to the National Cancer Institute, and (3) to prepare and characterize dispersions of coated and uncoated particles in any vehicle or solvent of interest to the National Cancer Institute.

Major Findings: Size fractions of carbon and the exoides of iron, aluminum, nickel and cobalt have been prepared and characterized. These have been successfully coated with benzo[a]pyrene (BP) and maintained in a stable form for periods up to three months. Suspensions of BP in saline and gelatin have been prepared and characterized. These preparations have been distributed to the institutions currently performing studies under the NCI respiratory carcinogenesis program.

Individual manufacturers' lots of BP have been found to behave differently when coated on iron oxide. Particle size and degree of attachment of BP to iron oxide in the coated product are the properties which seem to vary when different BP lots are used.

Significance to Biomedical Research and the Program of the Institute: The significance of the contract is that the various NCI contractors have a constant and dependable source of materials for their biological studies. This ensures that each contractor performing related experiments has standard uniform materials for intratracheal instillation experiments.

Proposed Course: To continue this service to NCI for as long as it is required.

Date Contract Initiated: June 10, 1971

Current Annual Level: $93,813

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-45608)

Title: Program for the Evaluation of Carcinogenic Risk of Chemicals to Man

Contractor's Project Directors: Dr. Lorenzo Tomatis
Dr. Claus Agthe

Project Officers (NCI): Dr. John A. Cooper II
Dr. Sidney Siegel

Objectives: The increasing number and quantity of chemicals introduced in our environment and their possible adverse effect, especially with regard to carcinogenicity, have called for a coordination of the world's effort to assess carcinogenic risk thus enabling preventive measures to be taken. The International Agency for Research on Cancer (IARC) with the support of the National Cancer Institute (NCI) is therefore, undertaking a program on the evaluation of carcinogenic risk of chemicals to man. The first aim of this program is to collect and evaluate the existing carcinogenicity data and disseminate such information in the form of monographs. This work is carried out with the help of international groups of experts. Monographs are prepared on chemicals for which there is significant human exposure and for which there is some published evidence of carcinogenicity in animals and/or man. They will be revised if and when new data and/or new principles for evaluation become available. The monographs have identified areas where further testing should be carried out and, because of the limited laboratory facilities for such testing, the second aim of the program is to coordinate testing through the survey of chemicals being tested for carcinogenicity. The information received from this survey is made available to all participating laboratories in the form of an information bulletin.

Major Findings: Up to March 1975 eight working groups have taken place covering a total of 196 substances. No conclusion could be reached on the possible carcinogenicity of 27 substances. For 18 chemicals the available data did not reveal a carcinogenic effect. Of the remaining substances, 41 showed some carcinogenic effect in experimental animals, 93 were definitely carcinogenic in experimental animals and 17 were evaluated as having been associated with cancer induction in man. Volume 8 concerning some aromatic azo dyes is in preparation. Further working groups will be convened to consider some aziridines, N-, S- and O-mustards and selenium in April 1975; some natural occurring toxins and antibiotics in October 1975 and epoxides, carbamates and related substances in December 1975. Four natural occurring toxins monographs, which were published in Volume 1, will be up-dated at the October meeting.

Two survey letters were sent out in Spring 1973 and 1974. The resulting information was issued in information bulletins 1-4. A further bulletin covering the 1974 survey will be published in May 1975, and a further survey started in Summmer 1975.

Significance to Biomedical Research and the Program of the Institute: (1) The IARC represents a unique resource as a virtue of its easy communication with

nations which sometimes could not otherwise freely exchange views. It is most likely that only such an international organization could establish either the registry of chemicals under test or the expert committees desirable for the production of the proposed monographs. (2) This activity will assist NCI in carrying out the responsibilities stated in the National Cancer Act of 1971 to "collect, analyze and disseminate all data useful in the prevention, diagnosis and treatment of cancer including the establishment of an international cancer research data bank to collect, catalogue, store and disseminate insofar as feasible, the results of cancer research undertaken in any country for the use of any person involved in cancer research in any country."

Proposed Course: The program will continue to operate on the basis of three working groups per year. In addition to the working groups proposed for 1975, three or four working groups are planned which will consider chemicals for which monographs have not yet been prepared and two or three meetings are foreseen to up-date groups of chemicals for which monographs already exist for some chemicals in these groups and adding some new ones. Candidate groups for such meetings are nitrosamines, aromatic amines and inorganic compounds. With regard to the survey of compounds being tested for carcinogenicity, this will be undertaken on a yearly basis and it is expected that one or two information bulletins will be issued as the result of one year's survey. The information bulletin will also be used to identify substances for which monographs will have to be prepared.

Date Contract Initiated: June 30, 1972

Current Annual Level: $175,000

JOHNS HOPKINS UNIVERSITY (NO1-CP-43288)

Title: Animal Pathology Support

Contractor's Project Directors: Dr. John D. Strandberg
Dr. Richard J. Montali

Project Officer (NCI): Dr. David Kaufman

Objectives: To provide animal pathology support to the Carcinogenesis Program, to investigate controversial lesions, to study histogenesis of induced neoplasms, to compare induced neoplasms with spontaneously occurring human and animal tumors and to provide training in tumor and experimental pathology.

Major Findings: Major support efforts have been provided in histopathologic examination of experimentally induced rat liver tumors and of diethylnitrosamine induced lung lesions in hamsters. Further pathology support has been provided in development of the bioassay pathology protocol, in evaluating the SNOP coding system used for diagnoses and in further developing an English language system for the storage and retrieval of pathologic diagnoses. Three spontaneous primate neoplasms have been identified and are under study.

In conjunction with other programs five individuals have been undergoing training in the field of comparative pathology.

Significance to Biomedical Research and the Program of the Institute: The work supported under this contract has aided in the establishment of uniform pathologic standards for use throughout the bioassay program. This will permit more valid interpretation and comparison of data from different sources. The development and adaptation of the SNOP coding system permits the pathologic data generated by many large studies to be easily assimilated, correlated, and reported in a wide variety of forms. The investigation of the histogenesis of chemically-induced neoplasms and comparison to naturally occurring tumors adds relevance to the studies under way and will hopefully allow insight into some of the basic mechanisms of preneoplastic changes. Training of pathologists in the field of tumor pathology will increase the restricted numbers of individuals skilled in this discipline to fill many positions now open.

Proposed Course: The work during the coming year will continue to involve training of pathologists and develop a working SNOP dictionary. In addition, further efforts will be directed toward the ultrastructural study of several lesions in an attempt to demonstrate their neoplastic nature and their cell of origin. One is the blood-filled lesion in the lungs caused by the intra-tracheal instillation of diethylnitrosamine. The other is a questionably granulomatous peritoneal reaction occurring in rats on another study. Further histopathology support will be provided and the files of animal tumor material at Johns Hopkins University will continue to be open to the use of the Carcinogenesis Program. The training program will continue as in the past.

Date Contract Initiated: February 1, 1974

Current Annual Level: $110,000

MEDIZINISCHE HOCHSCHULE HANNOVER (NO1-CP-12148)

Title: Development of a Lung Tumor Model with Large Wild European Hamsters (*Cricetus cricetus* L.)

Contractor's Project Director: Dr. Ulrich Mohr

Project Officers (NCI): Dr. Thomas Cameron
Dr. Norbert Page

Objectives: This contract was initiated to explore the possible usefulness of the European hamster (*Cricetus cricetus* L.) as an animal model for the induction of respiratory tract tumors morphologically similar to tumors found in man. The specific aspects are to (1) capture the wild hamsters and establish a breeding colony, (2) determine the incidence of spontaneous tumors, especially bronchogenic carcinomas, (3) test for sensitivity to induction of lung cancer by known carcinogens, and (4) attempt to develop other animal model uses for this animal.

Major Findings: While feral animals continue to be captured and utilized in some experiments, the development of a domestic colony continues with excellent success. The domestic colony now numbers almost 600 animals and is in the F_7 generation. Preliminary efforts are being made on a small scale to develop a melanistric line and an inbred strain. Twenty-eight animals have been successfully shipped to the NIH Animal Center at Poolesville to establish a colony.

The nitroso compounds study in the domestic animals has been continued and expanded. Evidence to date confirms that these animals are indeed sensitive to the carcinogenic effect and exposure results in high incidences of bronchogenic carcinoma and tumors of the nasal and oral cavities with a specific nitroso compound dependency.

An English language anatomical atlas of the European hamster has been prepared. It is now being edited for future publication. Efforts are being made to determine if the methods used to produce pancreatic tumors in the Syrian hamster will result in comparable results in the European.

Significance to Biomedical Research and the Program of the Institute: Regardless of the causative factors of the bronchogenic carcinoma, the availability of laboratory animals with this characteristic would be invaluable to cancer research. Other characteristics which would make this animal superior to the Syrian golden hamster are its larger size and its longer lifespan. At present there is no satisfactory animal model for study of the natural pathogenesis bronchogenic squamous cell carcinoma.

While this type of tumor can be induced in the Syrian golden hamster via intratracheal instillation of chemical carcinogens, they do not spontaneously develop these tumors. Consequently, it is necessary to have reservations in extrapolating data obtained with the Syrian hamster to man. In addition, the use of an animal that is subject to the spontaneous occurrence of squamous cell carcinoma might be more suitable to studies of lung cancer using a natural exposure method, i.e., inhalation of aerosols.

The program is of direct and intimate relevance to the lung cancer program. If this species can be bred and maintained as a laboratory animal, it would be a valuable animal for the bioassay programs as well as to study mechanisms and pathogenesis of spontaneous lung cancer.

Proposed Course: Initially it was necessary to capture and adapt this wild species to laboratory conditions and to develop a breeding program. The natural occurrence and incidence of diseases, especially lung tumors, has to be determined as this animal has a relatively longer lifespan than the commonly used laboratory rodents.

The chronic testing of known carcinogens by intratracheal instillation and inhalation will be pursued during the coming year.

In addition, efforts will continue to develop the pancreatic tumor model. Bronchioscopic techniques will be employed to observe and record the development of induced lung tumors.

Date Contract Initiated: June 22, 1971

Current Annual Level: $80,500

MIDWEST RESEARCH INSTITUTE (NO1-CP-23270)

Title: Analytical Chemistry Resource

Contractor's Project Director: Dr. Evelyn Murrill

Project Officers (NCI): Dr. Marcia Litwack
Dr. Larry Keefer

Objectives: The objective of this program is to serve as a state-of-the-art analytical facility for the Carcinogenesis Program. Its purpose is to precisely define the chemical nature of compounds in order to increase the accuracy and reliability of data obtained in carcinogenesis research. When the program began in June 1972, the scope of tasks included analysis of chemicals, chemical/feed mixtures and feeds for the Bioassay Operations Segment as well as specialized analysis for investigators in other segments. In July 1974, the program was divided into a general analytical resource (this program) and a resource specialized for the Bioassay Operations Segment.

Major Findings: Since June 1972, studies have been completed and reported on 148 batches of chemicals (121 different chemical substances). Ten have been analyzed in detail to identify and quantitate minor impurities, and 30 chemical mixes have been analyzed for stability or homogeneity. Routine characterization studies include chromatography, (TLC, GC, HPLC, etc.), elemental analysis, water analysis, and other assay methods dependent on the chemical (titration, voltammetry, mass spectrometry). Physical data include transition data (melting point and boiling point, refractive index, optical rotation, and spectra data (infrared, ultraviolet, visible and NMR). Specialized tasks have included synthesis, identity and assay of pure benzo-[a]pyrene; purification and assay studies of dimethylbenzanthracene; and a literature survey on the analytical methodology of the OSHA Carcinogens.

Significance of Biomedical Research and the Program of the Institute: The literature of carcinogenesis studies contains many examples of experiments which cannot be meaningful interpreted because the test substances had not been adequately characterized chemically. When such an experiment results in low tumor incidence, for example, the question will always remain as to whether the tumors observed might not have arisen from small concentrations of highly potent impurities, rather than from the allegedly weak potent nominal ingredient. Other questions of a chemical nature which are central to the interpretation of bioassay data include such problems as decomposition of the chemical in feed or intubation mixtures and levels of mycotoxins or pesticides in food and bedding. For these reasons it is essential that the Carcinogenesis Program have available to it a facility capable of characterizing those materials entered into the testing program, as well as determining the effectiveness of administration of these substances to the test animals.

Proposed Course: To continue the purchase, storage, and distribution of compounds used in the Carcinogenesis Program, and the analysis of bulk chemicals, chemical/feed mixtures, and feed.

Data Contract Initiated: June 28, 1972

Current Annual Level: $36,852

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CP-43255)

Title: Identification of Human Populations at Risk Due to Exposure to N-Nitroso Compounds

Contractor's Project Director: Dr. William J. Nicholson

Project Officer (NCI): Dr. Larry Keefer

Objectives: (1) To seek human populations which have had significant exposure to N-nitroso compounds, or high exposure to nitrates or nitrites which may promote the production of N-nitroso compounds in the human body; (2) to seek information on the extent of exposure for each specific population identified; (3) to develop protocols for epidemiologic investigations to assess increased tumor risk in appropriate groups that have been identified; and (4) to determine the feasibility of conducting studies based on the above protocols. Where possible, a sample of the defined population shall be studied or traced to determine the suitability of a complete study.

Major Findings: A variety of specific group exposures to N-nitroso compounds have been identified to date. These include the employees of chemical manufacturers, rubber industry employees, and students exposed during laboratory classwork. In each case the extent of the exposure, the number of individuals involved and the suitability of the group for future epidemiological study is being investigated. A small group of individuals, heavily exposed to dimethylnitrosamine 20 years ago during the development of a dimethylhydrazine rocket fuel facility, has also been identified and the majority of these individuals traced.

Significance to Biomedical Research and the Program of the Institute: N-Nitroso compounds have been shown to be acutely toxic and carcinogenic in animal studies, though little is known of their effect in humans. The disclosure that N-nitroso compounds can be found in food products and also synthesized in vivo, however, lends urgency to a determination of their effects in humans. The identification of industrial populations exposed to these compounds at levels much higher than those encountered in environmental circumstances offers the opportunity to demonstrating either the existence or absence of human health effects from such exposures.

Proposed Course: The activity under this contract has been terminated during the current fiscal year. An evaluation of possible alternative epidemiological investigations suggested during the course of this study will be conducted in the near future.

Date Contract Initiated: October 15, 1973

Current Annual Level: 0

NATIONAL ACADEMY OF SCIENCES, INSTITUTE OF LABORATORY ANIMAL RESOURCES (ILAR) (NO1-CP-45617)

Title: Partial Support for the Institute of Laboratory Animal Resources

Contractor's Project Director: Dr. Robert H. Yager

Project Officer (NCI): Dr. Thomas Cameron

Objectives: To develop guidelines for the care and use of the "nude" mouse in biomedical research

Major Findings: The ILAR has assembled a committee of experts familiar with the genetics, husbandry, and biomedical research uses of the "nude" mouse. The committee members are assembling data within their various fields of expertise for incorporation into guidelines.

Significance to Biomedical Research and the Program of the Institute: Since 1968, an enormous interest has developed in this mutant, primarily in immunology and cancer research. Its value for such research lies in the fact that it is considered to have, at most, a minute and grossly abnormal thymus and to lack thymus-derived (T) lymphocytes. Thus, it provides a source of B lymphocytes uncontaminated with T lymphocytes. Also, because the T lymphocytes are involved in cell-mediated responses, such as acceptance or rejection of skin and other foreign tissue grafts, the potential of this animal for cancer research is considerable. For example, it would be possible to study these tumors in vivo rather than in vitro. At present, there is preliminary evidence that this type of experimental procedure is possible. The importance of this animal for cancer research is obvious.

Proposed Course: Production of ILAR guidelines entitled "Guide for the Care and Use of the "Nude" (Thymus Deficent) Mouse in Biomedical Research." In the furtherance of the objectives of the animal resources activities of the Carcinogenesis Program, ILAR will continue its program for the collection and subsequent dissemination of well-substantiated information on genetically unique strains of laboratory models for particular human diseases. As a corollary to the accumulation of information on genotypic entities, the staff of ILAR maintains an information center that responds to inquiries on a multitude of aspects such as laboratory animal care, breeding, nutrition, management, and utilization. This information bank is readily available to the Carcinogenesis Program.

Date Contract Initiated: June 1, 1973

Current Annual Level: $10,000

NCI, FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) (NO1-CO-25423)

Project 9

Title: Preparation and Characterization of Carcinogens

Contractor's Project Manager: Dr. Walter Zielinski, Jr.

Project Monitor (NCI): Dr. Marcia D. Litwack

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

NCI, FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) (NO1-CO-25423)

Project 12

Title: Production of Inbred and Hybrid Laboratory Animal Strains

Contractor's Project Manager: Dr. Raymond Ediger

Project Monitor (NCI): Dr. Thomas Cameron

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

NCI, FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) (NO1-CO-25423)

Project 15

Title: Procedures for Safe Handling of Chemical Carcinogens

Contractor's Project Director: Dr. Eric Sansone

Project Monitor (NCI): Dr. Larry Keefer

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

NEW HAMPSHIRE, UNIVERSITY OF (NO1-CP-55675)

Title: Preparation of Various N-Nitroso Compounds

Contractor's Project Director: Dr. Robert Lyle

Project Officer (NCI): Dr. Larry Keefer

Objectives: (1) To determine effective and economical methods of synthesis of N-nitrosopyrrolidine, N-nitrosopiperdine and related derivatives of

nitrosamine heterocycles suitable for the preparation of carbon labeled compounds; (2) to prepare several unlabeled nitrosamines for inclusion in the carcinogenesis standard reference compound bank; and (3) to initiate other synthetic studies applicable to the preparation of labeled nitrosamines which might provide a better understanding of the biological mechanisms of action of N-nitrosamines.

Major Findings: The synthesis of nitrogen heterocycles by cyclization of dinitriles is feasible by catalytic hydrogenation and appears promising using complex natural hydrides. Thus introduction of 2 ring carbon labels from cyanide ion seems highly promising. A large scale preparation of the N-nitrosamine use of metal beta-phenethylethylamine is in progress.

The synthesis of N-nitroso-cis-2,6-dimethylpiperdine provides a convenient model for testing nitrosation reactions and functions as a substrate for studying the structure of complex of N-nitrosamines.

Significance to Biomedical Research and the Program of the Institute: The investigations made under this contract will contribute directly and indirectly to biological research on N-nitrosamines. These compounds are essential for an understanding of the metabolism and mode of carcinogenesis of nitrosamines.

The synthetic studies may provide clues about environmental synthesis of nitrosamines and structural information may suggest the nature of interaction of nitrosamines with biological systems.

Proposed Course: The synthetic studies will be continued to prepare nitrosamines labeled and unlabeled as needed for research of carcinogenesis.

Date Contract Initiated: January 31, 1975

Current Annual Level: $43,374

RESEARCH TRIANGLE INSTITUTE (NO1-CP-55671)

Title: Preparation of Various N-Nitroso Compounds

Contractor's Project Director: Dr. J.A. Kepler

Project Officer (NCI): Dr. Larry Keefer

Objectives: To synthesize adequate quantities of isotopically labeled N-nitrosamines that have been requested for investigations of the biological action of these compounds in initiating cancer. In addition, larger quantities of unlabeled N-nitrosamines will be prepared for reference purposes and supplying material for studies on the carcinogenicity of these compounds.

Major Findings: As this is a new contract, no major findings can be reported at this time.

Significance to Biomedical Research and the Program of the Institute: Nitrosamines are known to cause cancer. The nature and location of the tumor depends on the specific nitrosatable amine. Much work is going on throughout the world to understand the mechanism of carcinogenesis. The nitrosamines offer special opportunities to study the mechanism of carcinogenesis because of this specificity. The preparation of radio-labeled nitrosamines will be useful for such research.

Proposed Course: Compounds will be synthesized upon the request of NCI.

Date Contract Initiated: February 1, 1975

Current Annual Level: $40,000 (estimate)

STANFORD RESEARCH INSTITUTE (NO1-CP-55672)

Title: Preparation of Various N-Nitroso Compounds

Contractor's Project Director: Dr. Elmer J. Reist

Project Officer (NCI): Dr. Larry Keefer

Objectives: To synthesize adequate quantities of isotopically labeled N-nitrosamines that have been requested for investigations of the biological action of these compounds in initiating cancer. In addition, larger quantities of unlabeled N-nitrosamines will be prepared for reference purposes and supplying material for studies on the carcinogenicity of these compounds.

Major Findings: As this is a new contract, no major findings can be reported at this time.

Significance to Biomedical Research and the Program of the Institute: Nitrosamines are known to cause cancer. The nature and location of the tumor depends on the specific nitrosamine amine. Much work is going on throughout the world to understand the mechanism of carcinogenesis. The nitrosamines offer special opportunities to study the mechanism of carcinogenesis because of this specificity. The preparation of radio-labeled nitrosamines will be useful for such research.

Proposed Course: The immediate goal is to synthesize 4 kg of N-nitroso-N-butyl-4-hydroxybutylamine. Upon the request of NCI other compounds will be synthesized.

Date Contract Initiated: February 1, 1975

Current Annual Level: $40,000 (estimate)

STANFORD RESEARCH INSTITUTE (NO1-CP-33285)

Title: A Research Program to Acquire and Analyze Information on Chemicals That Impact on Man and His Environment

Contractor's Project Directors: Mr. Arthur A. McGee
Dr. Kirtland E. McCaleb

Project Officer (NCI): Dr. John A. Cooper II

Objectives: To develop a system to collect, analyze, and systematize information on the chemical description, production, distribution, and human exposure of chemicals with which the U.S. population comes in contact.

Major Findings: The present data base contains information on 3,500 chemicals in the following categories: intentional food additives, air pollutants, pesticide residues in food, water pollutants, proprietary drugs, soaps and detergents, prescription drugs, trade sales paints, and cosmetics. These categories are subdivided into 900 product types representing 18,000 chemical-product combinations.

The data are in computer-readable form and contain the following information:

Product:	Each Ingredient Chemical:
Product name	CAS number and name
Quantity available for exposure	Strength (percent) in each product
Exposure routes -- oral, dermal, respiratory, and parenteral	Degree of uncertainty associated with quantitative data
Exposure factor by route	References to data sources

A computerized chemical classification scheme has been developed and node assignments have been made for each of the chemicals for which exposure estimates have been made. As additional biological data become available, the practicality of making estimates of a molecule's carcinogenic potential based on structure will be further investigated.

Also in computer-readable form are data on 25,000 chemicals to which the U.S. population is most likely to be exposed. These chemicals were drawn from eighteen recognized sources of information on such products as cosmetics, food additives, medicinals, etc. For each of the 25,000 chemicals, the following information is available: chemical identifiers (CAS number, structural representation, chemical name, and synonyms), and the chemical structure classification nodes.

Under this project, information on chemical production, distribution, use, and exposure is being provided to the International Agency for Research on Cancer for inclusion in the IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man.

Developmental tasks under consideration include (1) analyzing trends in chemical production, exposure, and use; (2) further work in representing

structure-activity relationships; (3) refining exposure factors; (4) including data on high risk subpopulations; (5) enlarging the data base to include other chemical, physical, and biological information; (6) expanding the data base to include additional exposure categories and classes of chemicals; (7) reprogramming the data processing system to handle a larger data base and to respond to a greater range of inquiries; and (8) expand the system to serve other groups within NCI and other agencies, with regulatory and standard-setting responsibilities, concerned with health hazards arising from exposure to chemicals.

Significance to Biomedical Research and the Program of the Institute: The system being developed through this study provides information on many chemicals of interest both to the Carcinogenesis Program and to other programs concerned with the impact of chemicals on man.

Proposed Course: The project will continue as outlined above. It will continue to provide data useful for the selection of compounds to be introduced into the Carcinogenesis Bioassay Program. The information needs of the following activities will be determined and incorporated into the system where feasible: (1) activities in the overall Carcinogenesis Program and in other organizational elements within NCI, and (2) activities within other Government programs concerned with the health hazards arising from exposure to chemicals.

Date Contract Initiated: October 30, 1970

Current Annual Level: $430,357

TRACOR-JITCO, INC. (NO1-CP-33402)

Title: Production of 1972-1973 Volume of Survey of Compounds Which Have Been Tested for Carcinogenic Activity and Production of Machine Readable and Searchable Indexes of the PHS 149

Contractor's Project Director: Mr. Anthony Lee

Project Officer (NCI): Dr. Sidney Siegel

Objectives: To compile and publish data from the 1972-1973 scientific literature on the carcinogenic activity of chemicals in experimental animals. The resultant publication will be the 1972-1973 Volume of the PHS Publications Series No. 149, entitled Survey of Compounds Which Have Been Tested for Carcinogenic Activity.

Major Findings: This activity represents a continuation of the work carried out under NIH Contract 69-2086 and 71-2266, in which volumes covering the 1961-1967, 1968-1969, and 1970-1971 scientific literature were prepared.

The contractor has searched the scientific literature, selected appropriate documents, extracted and placed in a formal matrix specific data from these documents, and has indexed selected items from the extracted data. Indexes

were generated for (1) literature reference, (2) route of administration. (3) site of application, (4) animal species/strain, (5) tumor site, (6) vehicle used, (7) chemical names of compound administered, (8) Chemical Abstracts Registry (CAS) number of the compound administered, (9) molecular formula of compound administered, and (10) Wiswesser line notation of the compound administered.

Cumulative indexes of the first six PHS 149 Volumes have been converted into series of computer readable data base files.

Significance to Biomedical Research and the Program of the Institute: The 1972-1973 Volume of PHS Publication No. 149 will bring the series of volumes up to date and provide a tool useful in searching the chemical carcinogenesis literature. The conversion of the indexes to a machine searchable data base will enhance the utility of this information.

Proposed Course: The contract has terminated and the material for the volume covering information for 1972-1973 is now in press.

Date Contract Initiated: June 30, 1973

Current Annual Level: $114,366

WOLF RESEARCH AND DEVELOPMENT CORPORATION (NO1-CP-43257)

Title: Carcinogenesis Bioassay Data System (CBDS) Operational Support

Contractor's Project Director: Mr. Dalton Tidwell

Project Officer (NCI): Mr. Joseph Sberro

Objectives: This project links data and information among the CBDS bioassay contractors, the Bioassay Operations Segment, the Carcinogen Bioassay and Program Resources Branch, and the CBDS computer subsystem implemented through the Division of Computer Research and Technology (DCRT), NIH. The contractor operates a subsystem to accept, log, and process data collection forms submitted by the scientific research organizations participating in the CBDS. Trained and qualified staff operate this subsystem with functions including: forms control, editing and coding, maintenance and development of a microfilmed data base; operation of an animal inventory; maintenance of automated files for contract information and chemical information; a comprehensive educational program on the development, operation, maintenance, and use of the CBDS to include site visits and data seminars; and preliminary statistical and pathology analysis.

Major Findings: An operational system to control and process CBDS bioassay contractor data from initial data entry to updating the CBDS data base, creating microfiche records, and preparing Preliminary Analysis Reports for all data processed has been developed and documented in collaboration with the Carcinogenesis Program and with DCRT, NIH. A CBDS Chemical File has been developed to maintain current chemical information for all chemicals on

bioassay testing. A CBDS Contract File has been developed to display administrative, chemical, and treatment information for all agents being tested by bioassay contractors. As an ongoing effort, support is provided in a collaborative study with the International Agency for Research on Cancer (IARC) registry program. Information on chemicals being tested for carcinogenicity, under NCI contract, is being compiled and routinely submitted to IARC. During the current contract period, over 160,000 data collection forms were processed and entered into the CBDS data base and recorded on microfiche. Preliminary Analysis Reports for numerous chemicals have been prepared and the results reported to NCI to assist in evaluation of the bioassays. These data are available for analysis by NCI investigators and others by special arrangement.

Significance to Biomedical Research and the Program of the Institute: Data collected from bioassay studies are important in formulating public policy pertaining to the continued use of the agents tested. It is important that the results of these studies be properly recorded and analyzed. The CBDS will provide for the reporting of these data.

Proposed Course: The subsystem implemented by the contractor is functioning and is proposed to continue in operation through the data collection and analysis phase of the carcinogenesis bioassay study.

Date Contract Initiated: June 26, 1972

Current Annual Level: $298,839

SUMMARY REPORT

LUNG CANCER SEGMENT

July 1, 1974 through June 30, 1975

The Lung Cancer Segment is devoted to studies of the pathogenesis of lung cancer and its prevention. The ultimate goal of the program is the prevention of development of lung cancer in man. A variety of experimental techniques are used in many investigations, ranging from long-term animal studies to short-term morphological and biochemical experiments. All of these efforts are coordinated to establish a correlation between animal models and human lung cancer, and to develop a targeted approach to the inhibition or prevention of this most prevalent form of fatal cancer in man. The need for such a program to lessen the incidence of this widespread, lethal disease scarcely requires further comment. During the past year major investigations in the areas of in vitro carcinogenesis in respiratory epithelium, in carcinogen metabolism in respiratory epithelium, and in human respiratory tract tissue have further developed. Significant accomplishments of the entire program during the past year are described below.

At the Oak Ridge National Laboratory (ERDA-NCI Interagency Agreement [YO1-CP-50200]) the pathogenesis of lung cancer is being investigated in a number of long-term animal studies that are of direct relevance to the human disease. The potential cocarcinogenicity of several irritant gases, that are common combustion products (such as nitrogen dioxide) or are prevalent in the gas phase of tobacco smoke (such as formaldehyde), is being tested in rat and hamster lung tumor models. The toxic response of the respiratory tract epithelium to these irritants is also under investigation. Preliminary data from one benzo[a]pyrene-nitrogen dioxide study suggest a cocarcinogenic effect of this irritant gas. Studies have been performed to investigate the effects of both high and low vitamin A intake on the induction and growth of lung cancer. It has been confirmed that administration of high doses of vitamin A to rats partially protects the respiratory tract epithelium from the tumorigenic effects of a polynuclear hydrocarbon such as methylcholanthrene, while maintenance of rats on a low vitamin A intake results in an increased susceptibility to lung cancer induction. Two important systems for respiratory tract carcinogenesis and anti-carcinogenesis studies have recently been developed. The first model is a new system for grafting tracheas into recipient animals; carcinomas can be induced in the transplants by suitable application of carcinogen. This model has been extensively characterized and further extended to a variety of tissues. The second new system allows tumor induction in the intact hamster at a well-known and predetermined segment of the trachea by repeated application of carcinogen to this defined region. This new animal model will considerably facilitate detailed morphogenesis studies and has already been used in a study which for the first time correlates the histology of a given lesion with its own exfoliated cells.

At the New York University Medical Center (NO1-CP-33260), the major emphasis is on the identification of hazardous materials which may pose a serious threat to man as respiratory carcinogens. Great effort has been spent in the

design and establishment of an inhalation chamber facility which can accomodate over 1000 animals. Systems for the generation of aerosols, vapors, and industrial dusts have been developed for testing the carcinogenicity of many substances by the inhalation route. Among the important substances which have been found to be carcinogenic or cocarcinogenic for the respiratory tract are calcium chromate dusts, sulfur dioxide, dusts formed from polyurethane foam(workers in the building trades industry may be exposed to this type of dust), and bis-chloromethyl ether, a common industrial chemical. Results in long-term animal studies still in progress indicate that nitrogen dioxide is cocarcinogenic in rats after combined inhalation exposures to benzo[a]pyrene-nitrogen dioxide; and that inhaled croton oil is a promoting agent for benzo-[a]pyrene in the hamster respiratory tract. Chronic inhalation studies have begun on chrome process materials actually used in commercial chromium refining; chronic studies are being developed for further studies of polyurethane foam. Results obtained from this contract have already been used by the Occupational Safety and Health Administration in the Department of Labor to help set standards of exposure for industrial workers to occupational carcinogens.

At the Harvard University School of Public Health (NO1-CP-33273), the synergism between radiation and chemical carcinogens in causing lung cancer is being studied. Such synergism has been implicated in the high incidence of lung cancer in uranium miners.

Animal studies in the present contract have shown that intratracheally-administered polonium-210 and benzo[a]pyrene reach different sites in the lungs; benzo[a]pyrene, primarily the upper airway epithelium and ^{210}Po, the peripheral lung. Despite this fact, the two carcinogens have been found to act synergistically in producing peripheral tumors. This important finding is being investigated further in several new series of investigations: (1) long-term animal studies of synergism in which both carcinogens reach the same peripheral cell population; (2) interaction studies between inhaled radon daughters and benzo[a]pyrene administered intratracheally; and (3) studies carried out in cell culture examining various parameters of malignant transformation under varying conditions of carcinogen interaction. A synergistic action between x-rays and benzo[a]pyrene has already been observed in this cell culture system, which was dependent upon the sequence and time interval between treatments. The roles of cell density and of cell proliferation in this system are actively investigated.

At the IIT Research Institute (NO1-CP-23292), genetic as well as environmental factors are being assessed for their influence on development of lung cancer in experimental animals. The carcinogenicity of benzo[a]pyrene for the respiratory tract of different strains of inbred hamsters is being measured; results to date suggest that inbred strains 15.16 and 1.5 are more resistant to the effects of this respiratory carcinogen than are strains 87.20 and NIH outbred hamsters. This study has human relevance, for if biochemical and cellular differences can be identified in these animal experiments, significant leads may be found for eventual assessment of the risk of individual human beings to exposure to respiratory carcinogens. Another important factor which may modify host response to respiratory carcinogens is viral or bacterial infection of the respiratory epithelium. Previous work at IITRI had

demonstrated that APR/8 influenze virus administered intranasally and followed by a secondary insult of gelatin-saline produced a hyperplastic bronchial and bronchiolar epithelium. Recent studies have characterized the kinetics of this proliferative response and demonstrated the feasibility of administering a carcinogen such as benzo[a]pyrene before, during, or after maximal hyperplasia. These studies may help determine whether viral infection is a state of increased susceptibility to carcinogens. In a major long term study, 13-cis-retinoic acid, a less toxic synthetic analog of vitamin A, has been shown to prevent the development of lung cancer in animals that have been previously treated with the carcinogen, benzo[a]pyrene. These results are important since the practical usefulness of natural vitamin A in preventing the development of lung cancer is limited by its intrinsic toxicity.

Since many projects in the lung cancer program involve long-term animal studies in defined models of lung carcinogenesis, a contract was initiated this year for the supply of hamsters treated with respiratory carcinogens (IIT Research Institute [NO1-CP-43289]). This contract will prepare and deliver to the program Syrian golden hamsters treated under protocols leading to carefully controlled conditions for the intiation of preneoplastic lesions and lung tumors. The various treatment regimens and periods of treatment are used to produce a variety of preneoplastic lesions. It is anticipated that this project will importantly contribute to studies of induction and inhibition of respiratory cancers.

The results of many of the above animal studies are being utilized in several human studies in the Lung Cancer Segment. In the contract with St. Mary's Hospital (NO1-CP-23233), an intensive effort is being conducted to improve the technique of sputum cytology and to use it effectively for earlier diagnosis of lung cancer in man. A large group of uranium miners is being followed with sputum cytology, in order to achieve the earliest possible diagnosis of lung cancer in this population of workers who are at such high risk for development of lung cancer. Emphasis is also given to study of premalignant cytological cell pattern changes which, over a long period of time, show gradual development from benign to malignant cells. Such studies suggest that epidermoid and related malignant lesions of the lung develop very gradually over a relatively long period of time remaining as in situ carcinoma, on the average, for more than four years.

Since a significantly large number of deaths from lung cancer in man are caused by oat cell carcinoma (which is a particularly invasive and metastatic type of tumor), the Stanford University School of Medicine (NO1-CP-43295) is investigating the morphological and biochemical properties of this type of cancer. A leading hypothesis is that oat cell carcinomas arise from a specific type of cell (K-type cells) in the respiratory tract. Studies in this contract are aimed at identifying the unique morphological and biochemical properties of the K-type cells, as well as the factors or mechanisms involved in the neoplastic transformation of these cells. A large number of human oat cell tumors have been studied cytologically and ultrastructurally, and cell lines from them have been established and examined. The K-cell is neither argentaffine nor readily stainable with argyrophilic techniques, as is its counterpart, the intestinal argentaffine cell. It has been possible, however, to demonstrate the K-cell in rabbit lung by a modified silver stain.

Another project to study human tissue is in progress at the University of Maryland (NO1-CP-43237). Human respiratory epithelium is being characterized by electron microscopic and cytochemical techniques, and is also being placed in organ culture in order to study its response to carcinogens. It has been established that explants of human bronchi can be maintained for as long as six months in organ culture, with the bronchial epithelium showing non-keratinizing squamous metaplasia after two to four months. Explants incorporate radioactive precursors into RNA, DNA and protein. Collaborative experiments have shown the important result that these human bronchial epithelial cells in organ culture bind several polycyclic hydrocarbons in both nucleus and cytoplasm of all major epithelial cell types, as shown by radioautography, and to their DNA as shown by equilibrium density gradient banding in cesium chloride. Binding is decreased by 7,8-benzoflavone and butylatedhydroxy-toluene. This entire project will give valuable correlations between animal studies and the problem of lung cancer in man.

To further study the progression of preneoplastic lesions that will be induced in the previous project, a new program is underway at Litton Bionetics, Inc., (NO1-CP-43274). Human bronchial epithelial tissues bearing preneoplastic lesions will be transplanted and maintained in vivo as xenografts in the athymic nude mouse to determine whether such lesions have the potential to progress toward invasive malignancy. It has been established that the athymic nude mouse will support relatively long-term survival of human bronchial tissue xenografts.

To effectively pursue many of the above studies on lung tissue from both man and animals, improved cytological methods, such as radioautography, are being conducted at the VA Hospital in Tampa (YO1-CP-55625). The technique of light microscopic radioautography of the respiratory tract has been improved, so that the actual amount of radioactivity in cellular and subcellular compartments of respiratory epithelium can be quantitatively measured. It has been found previously that intratracheal administration of benzo[a]pyrene, as well as vitamin A deficiency, causes an increase in cellular proliferation in respiratory epithelium, as quantitated by thymidine labelling. In addition, it has been found that vitamin A deficiency causes loss of ciliated cells and an increase in the number of basal cells in respiratory epithelium. High doses of vitamin A cause an increase in ciliated cells.

The in vivo binding of benzo[a]pyrene in hamster trachea following a single intratracheal intubation has shown both nuclear and cytoplasmic binding in tracheal cell types. Binding persisted throughout a four-week observation period. Vitamin A-deficient hamsters showed significantly greater in vivo binding of benzo[a]pyrene in both non-metaplastic respiratory epithelium and in areas of squamous metaplasia.

Investigations are pursued in two projects devoted entirely to the study of respiratory carcinogenesis in vitro. At the State University of New York at Stony Brook (NO1-CP-33361), methods have been developed for large-scale, long-term culture of rat tracheal tissue. A protocol for exposure of the cultures to benzo[a]pyrene has been developed which results in areas of focal hyperplasia having a high level of thymidine incorporation. These focal hyperplasias are studied for persistence, response to promoters, reversibility

with vitamin A, and growth characteristics in a host. Work has established that Wistar Lewis rats are suitable as both donor and host in studies testing for the malignant potential of tissue altered by carcinogen in organ culture.

In the second project, organ culture studies at the University of Vermont (NO1-CP-33360) have systematically explored various chemically-defined media and the effects of serum, insulin, vitamin A and adrenal cortocosteroids on long-term cultures of hamster tracheal epithelium. Proliferative epithelial changes were found in organ cultures maintained in "complex" media containing serum whereas use of these media in the absence of serum or vitamin A produced varying degrees of squamous metaplasia and keratinization. A procedure for applying varying amounts of carcinogenic hydrocarbons to the epithelial surface in culture by use of glass or Lycra fibers dipped in carcinogen solutions has been developed. This procedure has allowed a systematic evaluation of epithelial changes at a defined site over a range of time periods. Carcinogen-treated and control tracheal rings maintained in organ culture for up to six months have been transplanted back to intact animals to see if they will develop into tumors. Both of these projects should yield valuable information about factors that either promote or inhibit the development of lung cancer. There is good communication between the two projects, and the joint effort should be mutually helpful.

Since it is well established that many carcinogens (including those which affect the lung) must be metabolically activated before they can exert their carcinogenic effects, a major effort has been developed in this area. Such research has long-term potential to find effective means to block the conversion of inhaled or ingested carcinogens to metabolically active forms, and thus to inhibit the development of lung cancer. Three contracts to study polycyclic hydrocarbon metabolism in the respiratory tract have been initiated.

At the University of Minnesota (NO1-CP-33364), efforts are focused on the role of anti-oxidants, such as butylated hydroxyanisole (BHA), in the inhibition of pulmonary neoplasia resulting from administration of polycyclic hydrocarbons. It has been found that BHA alters the metabolism of benzo[a]-pyrene (BP) by the microsomal mixed function oxidase system under conditions which inhibit BP-carcinogenesis. Use of microsomes from mice fed BHA results in significantly less binding of BP metabolites to DNA than with control microsomes. Binding spectra with control microsomes and microsomes from BHA-fed mice indicate the presence of an altered cytochrome P-450. Such an altered cytochrome P-450 could contribute to the observed decrease in BP metabolite-DNA binding.

At the University of Stockholm (NO1-CP-33363), efforts are directed at defining the relationship between the microsomal enzyme system, aryl hydrocarbon mono-oxygenase, and lung cancer. A sensitive and convenient radioactive assay for benzpyrene mono-oxygenase has been developed; and a new fluorometric assay for this enzyme has been applied to rat lung tissue to investigate its kinetics and inducibility. Important studies of benzpyrene metabolism by isolated, intact rat hepatocytes have been carried out, including kinetics, inhibition, effects of inducers and nature of products. A method for removing hemoglobin from lung microsomes by sepharose

chromatography has been developed. This has made possible improved spectral characterization of cytochrome P-450 in lung microsomes. The important isolation and characterization of a purified preparation of lung cytochrome P-450 itself has been achieved.

Since the aryl hydrocarbon mono-oxygenase system is an extremely complex linkage of various molecular components, the investigators at the University of Texas (Southwestern Medical School at Dallas)(NO1-CP-33362) are analyzing the role of the various molecular components in lung microsomes. Several chemical and immuno-chemical inhibitors have been used to investigate the normal and carcinogen-induced cytochrome P-450-dependent mono-oxygenases. Use of chemical inhibitors of BP hydroxylase or biphenyl hydroxylase show that the lung contains a constitutive aromatic hydroxylase which is quite active, but does not hydroxylate BP well. A 3-methylcholanthrene inducible aromatic hydroxylase exists which is P-450-dependent and which appears to hydroxylate BP but does not hydroxylate biphenyl. These results indicate that two types of cytochrome P-450-dependent aromatic hydroxylases exist in lung, but only the inducible hydroxylase appears to be capable of metabolizing BP.

It is apparent that a great deal of important basic biochemistry remains to be done on this problem, but the results are of great importance to our understanding of the initiation of lung cancer in man. Meetings are held frequently by the three groups of investigators working on this entire problem of polycyclic hydrocarbon metabolism, with a maximal sharing of information.

Since vitamin A has such a profound effect on cellular differentiation in respiratory epithelium, and since it has been shown to be capable of inhibiting carcinogenesis in this epithelium, the biochemical bases of these phenomena have been investigated, with the long-term goal of achieving more effective use of vitamin A or vitamin A analogs in cancer prevention. The role of vitamin A in controlling the synthesis of specific glycoproteins in respiratory epithelial cells has been studied at the Massachusetts Institute of Technology (NO1-CP-43253). It has been found that vitamin A is required for the synthesis of specific glycopeptides in these cells, both in the whole animal and in organ culture. Various parameters that affect the ability of vitamin A to block the development of lung cancer in the experimental animal were studied in this project.

Since the intrinsic toxicity of natural vitamin A is a limiting factor in its usefulness in cancer prophylaxis, studies are underway to identify new synthetic analogs of vitamin A that will be more potent and less toxic than the parent substance. At the Southern Research Institute (NO1-CP-22064), such potential analogs are being evaluated, by both morphological and biochemical techniques. These assays have the important feature of providing rapid test data on unknown compounds. The morphological effects are being evaluated in organ cultures of chick skin and mouse prostate, while biochemical studies have employed tissue from both mice and hamsters (liver and lung). In the organ culture assays, several modifications of the ring portion of the natural vitamin A molecule have been found to be more potent than the parent compound in inducing mucus metaplasia of chick skin. The organ culture system for mouse prostatic tissue has become firmly established, and has

already shown that increased proliferative activity in explants cultured in the presence of 3-methylcholanthrene can be inhibited by retinoic acid. The vitamin A compounds have been evaluated as inhibitors of microsomal mixed function oxidases from liver and lung tissues of mice and hamsters. Some of the compounds are potent inhibitors of the enzymatic oxidation of BP and other carcinogenic polycyclic hydrocarbons to products which become tightly bound to reaction components. Alterations in the metabolism of polycyclic hydrocarbons by vitamin A compounds may be related to their anticarcinogenic action.

In summary, the Lung Cancer Segment is pursuing a coordinated program of studies at the human, animal, cellular, and molecular level to investigate the factors which are involved in the cause and prevention of lung cancer. It is hoped that within this coordinated framework there will be the most rapid dissemination and utilization of new information obtained at the basic levels, and that we thus may hopefully be able to shorten the time before we achieve the desired goal of prevention of lung cancer in man.

CONTRACT NARRATIVES

LUNG CANCER SEGMENT

July 1, 1974 through June 30, 1975

ERDA-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) (Y01-CP-50200)

Title: NCI-ERDA Carcinogenesis Program

Contractor's Project Director: Dr. Francis T. Kenney

Project Officer (NCI): Dr. Allen Heim

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

Current Annual Level (Lung): $776,500

HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH (N01-CP-33273)

Title: Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens

Contractor's Project Director: Dr. John B. Little

Project Officers (NCI): Dr. Carl E. Smith
Dr. David Kaufman

Objectives: To examine some of the factors which influence the carcinogenic response to respiratory carcinogens and to determine in particular whether a synergistic or potentiating effect exists between alpha radiation and chemical carcinogens in the production of lung cancer. Polonium-210 (^{210}Po) will be used as a local source of alpha radiation, benzo[a]pyrene (BP) as a local chemical carcinogen and diethylnitrosamine (DEN) as a systemic carcinogen.

Major Findings: Lung cancer has been induced in hamsters by the intratracheal instillation of either ^{210}Po or BP adsorbed onto hematite particles. No tumors occurred in untreated or hematite-treated control animals. Distribution studies with polonium either in saline or on various concentrations of hematite particles indicate that the uniform distribution of alpha-emitting radionuclides as occurs following saline instillation is equally carcinogenic as is the non-uniform distribution or "hot-spots" seen with the particulate instillation. Localization studies have shown that intratracheally administered ^{210}Po delivers its major dose to the epithelium of the distal airways, as does BP administered in a gelatin-saline suspension. BP administered on hematite carrier particles, however, localizes largely in the tracheal and bronchial epithelium. These are the respective regions where the malignant tumors induced by each agent arise. Tumors have been induced in about 10% of hamsters receiving alpha radiation doses of only 15-75 rads from ^{210}Po.

A significant synergistic effect has been demonstrated between low doses of ^{210}Po and BP administered sequentially; a similar synergistic interaction has been demonstrated in an in vitro transformation system. Preliminary data suggest that cell proliferation between exposures may play an important role in such interactions.

Significance to Biomedical Research and the Program of the Institute: The proposed studies are addressed to a very critical problem in the evaluation of human lung cancer hazards which may result from the combined effects of radiation and chemical carcinogens. Synergistic relationships between alpha radiation and chemical carcinogens have been implicated in the high incidence of occupational lung cancer in man (uranium, hematite, and flurospar miners). Previous studies have shown that ^{210}Po, a component of tobacco smoke, accumulates in the bronchial wall, especially the segmental bifurcations of smokers. This is a region where a high percentage of the bronchial carcinomas are found. The possible role of ^{210}Po, acting as a cofactor along with other chemicals in tobacco smoke, needs clarification. Results from this project could have a direct bearing on the evaluation of lung cancer hazards in man, and on future developments in the whole Lung Cancer Program.

Proposed Course: Studies will be continued to assess synergistic effects between BP and ^{210}Po administered simultaneously and sequentially, and to evaluate the importance of cell proliferation and radiation dose-rate in such interactions. Techniques of administration will be sought which will deliver both carcinogens to the same site in the lungs. We will continue parallel studies with an in vitro transformation system, which may allow the identification of particularly hazardous combinations of respiratory carcinogens.

Date Contract Initiated: May 30, 1971

Current Annual Level: $140,000

IIT RESEARCH INSTITUTE (NO1-CP-43289)

Title: Supply of Hamsters Treated with Respiratory Carcinogens for NCI Carcinogenesis Program

Contractor's Project Director: Dr. Mary Henry

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: To prepare and deliver carcinogen-treated Syrian golden hamsters to be used as animal models for respiratory tract carcinogenesis studies by NCI.

Major Findings: Administration of carcinogens by the intratracheal route is done with well-defined techniques of chemical preparation and handling, instillation procedure and animal care. These protocols lead to carefully controlled conditions for the initiation of preneoplastic lesions and lung tumors. The treatment regimens include weekly treatment for eight to ten weeks with benzo[a]pyrene/ferric oxide or carbon, four or five weekly

treatments with the carcinogen dust mixture alternating with four or five weekly treatments of N-nitrosomethylurea. The various treatment regimens and periods of treatment are used to produce a variety of preneoplastic lesions. Different dust entities are used to vary the total exposure time of the trachea to the carcinogen. Health status of the animals is monitored by measurements of antibody serum levels to viral agents, bacteria in lung and organ culture of trachea.

Significance to Biomedical Research and the Program of the Institute: This contract is one of an integrated program examining facets of the induction of respiratory tract tumors in animal models and the phenomenon whereby vitamin A inhibits respiratory carcinogenesis. This is of great significance for the problem of lung cancer in man as well as for the problem of inhibition of carcinogenesis in the experimental animal.

Proposed Course: To continue the intratracheal instillation of carcinogens with current protocols and examine the production of preneoplastic lesions by other carcinogens with alternate dosage regimens and protocols.

Date Contract Initiated: May 30, 1974

Current Annual Level: $151,000

IIT RESEARCH INSTITUTE (NO1-CP-23292)

Title: Susceptibility States and Modulating Factors in Respiratory Carcinogenesis

Contractor's Project Director: Dr. Curtis D. Port

Project Officers (NCI): Dr. David Kaufman
Dr. Michael Sporn

Objectives: The principal objective of this program is to investigate factors that may affect the susceptibility of the respiratory epithelium to a carcinogenic insult as well as to study those factors which may modulate the carcinogenic response. Although the emphasis of the program will continue to be directed toward respiratory tract carcinogenesis, other epithelial components that may respond in a manner similar to that of the respiratory epithelium will also be investigated. Thus, the anticarcinogenicity of several vitamin A analogs will be investigated in a variety of epithelial tumor models, namely the DMBA-induced rat mammary tumor, the nitrosamine-induced esophageal tumor, the nitrosamine-induced rat urinary bladder tumor, and the nitrosamine-induced pancreatic tumor.

Major Findings: Previous work has demonstrated that APR/8 hamster-adapted influenza virus administered intranasally and followed by an additional insult of gelatin-saline produced a hyperplastic bronchial and bronchiolar epithelium. Studies were conducted to determine the time relationships between virus infection and a secondary tracheal insult on the development of this epithelial hyperplasia, which may be a facet in the expression of respiratory cancer.

These studies have given a method of inducing an epithelial response that is reproducible and easily obtained with all of the respiratory epithelia involved. The kinetics of the proliferative response in this system are such that a carcinogen can be administered before, during, or after maximal hyperplasia. A well-defined epithelial proliferative response now makes possible better experiments to investigate the relationship between epithelial proliferation and carcinogenesis. These studies may help determine whether viral infection is a state of increased susceptibility to carcinogens.

In a program in vitamin A and cancer prevention, a less toxic retinoid, 13-cis retinoic acid, was evaluated for its usefulness in preventing lung cancer in hamsters. The number of respiratory tract tumors and lesions in the treated groups suggest that 13-cis retinoic acid influences the development of carcinoma. The data also suggest that 13-cis retinoic acid does not affect the total number of lesions, but inhibits the progression of lesions to squamous carcinoma of the lung. The 13-cis retinoic acid was generously supplied by Hoffman La-Roche, Inc.

A study was conducted to identify carcinogen susceptible and resistant strains of inbred hamsters, using proven carcinogen regimens. The identification of such susceptible or resistant strains would enable further studies of carcinogen metabolism in relation to tumor yield in a defined tumor model. The results suggest that inbred strains 15.16 and 1.5 are more resistant to the effects of a respiratory carcinogen than are strains 87.20 and NIH outbred hamsters. Approximately 10 percent of the animals remain on test and histopathologic examination of these remaining animals should either accentuate or minimize these differences.

States of vitamin A deficiency have been found to be associated with a high rate of cellular proliferation and carcinogen binding in respiratory epithelium. This finding suggests that there may be a synergistic effect between vitamin A deficiency and carcinogen action in respiratory epithelia. Preliminary studies were conducted to determine the vitamin-A-acid dosage to permit hamsters to attain the age for, and withstand a series of, BP-hematite instillations. Although 10 μg of vitamin A acid given two times weekly is sufficient to sustain life, 90 μg of vitamin A acid also two times weekly is required for hamsters to withstand a series of BP-hematite instillations and live. Based upon the results of these preliminary studies, a more detailed tumorigenesis study may be initiated to determine both the susceptibility of the respiratory epithelium to BP, and the modulating effects of vitamin A deficiency on this process.

Significance to Biomedical Research and the Program of the Institute: This contract is one of an integrated program of contracts examining facets of the induction of respiratory tract tumors in animal models. Studies in this contract are intended to identify situations which place experimental animals at reduced or increased risk to the development of respiratory tumors. Identification of situations which increase carcinogenic risk may be exploitable by preventive measures. Situations of reduced risk may be exploitable as prophylaxis. By analogy with these animal model studies, exploitable situations in human cancer may be suggested.

Proposed Course: The virus-induced epithelial hyperplasia, 13-cis retinoic acid, vitamin A deficiency, and inbred hamster studies will be continued. In addition, studies will be initiated in the use of nonmetabolizable carcinogens that could be delivered intratracheally in an accurate and reproducible dose. These carcinogens would be highly desirable in evaluating the influence of vitamin A analogs on respiratory tract carcinogenesis. Furthermore, since vitamin A affects many epithelial tissues, it is of importance to define whether an anti-carcinogenic response in tissues, other than those of the respiratory tract, can be usefully used to predict for activity in preventing lung cancer.

Date Contract Initiated: June 26, 1972

Current Annual Level: $399,000

LITTON BIONETICS, INC. (NO1-CP-43274)

Title: Studies of Carcinogenesis in Human Bronchial Tissues

Contractor's Project Director: Dr. Marion Valerio

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: To further develop an animal model, the nude athymic mouse and immunosuppressed thymectomized hamster, for long-term survival of xenografts in order to study the reaction of human tissues (bronchus) exposed to various carcinogens and anticarcinogens.

Major Findings: There are major husbandry problems involved in the long-term survival of nude mice necessitating facility renovation. It was established that the nude athymic mouse will support relatively long-term survival of human bronchial tissue xenografts. The use of antilymphocyte serum allows xenografts to survive at least one month in neonatally thymectomized hamsters.

Significance to Biomedical Research and the Program of the Institute: The establishment of an animal host for human bronchial tissue xenografts which have been treated with carcinogens and/or anticarcinogens in vitro and/or in vivo will provide a model more predictive of the effects of carcinogens and anticarcinogens on human tissues than extrapolation from an animal tumor or tissue culture model system.

Proposed Course: Proceed to implant xenografts of human bronchial tissue in the athymic nude mouse to develop the long-term (up to one year) model desired. Given the model, the effects of various carcinogens, and anticarcinogens will be tested using this system.

Date Contract Initiated: February 1, 1974

Current Annual Level: $111,205

MARYLAND, UNIVERSITY OF (NO1-CP-43237)

Title: Studies on Normal, Premalignant and Malignant Tracheobronchial Epithelium in Humans

Contractor's Project Director: Dr. Benjamin F. Trump

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: (1) Morphologic and cytochemical studies of human bronchial epithelium in normal, premalignant and malignant states; (2) morphological and cytochemical studies of bronchial epithelium in normal, premalignant and malignant states maintained in organ culture; and (3) xenotransplantation of normal, premalignant, and malignant human bronchial epithelium.

Major Findings: (1) Numerous ultrastructural cytochemical and light microscopic studies have been made of the normal and reactive epithelium. A tentative new classification of cells and their relationships has been put forward. Cytochemical studies emphasize relationship of small mucus granule cells to squamous metaplasia. (2) Conditions have been established to obtain, store and transport human bronchial epithelium and to culture it for long periods of time up to six months. Studies have been made of carcinogen metabolism including DNA binding. (3) Feasibility of xenotransplantation of human bronchial epithelium into nude mice has been established and studies are now in progress on its response to carcinogens.

Significance to Biomedical Research and the Program of the Institute: The importance of this project lies in the fact that the nature of premalignant changes in the human bronchus are not understood and little is known concerning carcinogen binding and the response to carcinogens of human tissues. These studies, it is hoped, will not only be of significance in understanding carcinogenesis in the bronchus, but also establish a model for similar studies in other organ systems.

Proposed Course: (1) To further determine the nature of the cellular response to carcinogens in the human bronchus and the kinetics of cell proliferation, especially with regard to premalignant lesions; (2) to establish a classification of lung tumors based on this consideration; and (3) to examine the metabolic and morphologic response of human bronchus to carcinogens in vitro and in vivo and to correlate this response with smoking and occupational histories, as well as with carcinogen metabolism by other cells such as peripheral blood monocytes.

Date Contract Initiated: October 1, 1973

Current Annual Level: $113,259

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CP-43253)

Title: Role of Vitamin A in the Control of Differentiation and Carcinogenesis in the Respiratory Tract

Contractor's Project Director: Dr. Paul M. Newberne

Project Officer (NCI): Dr. Michael Sporn

Objectives: The specific aims of this project are the following: (1) to investigate the histological response of respiratory epithelium to benzpyrene carcinogenesis with retinol at different dosages and times; and (2) to correlate tumor incidence and development with vitamin A status.

Major Findings: Male, Syrian hamsters fed a commercial diet were given a series of 12 intratracheal instillations of 3 mg benzo[a]pyrene (BP) adherent to 3 mg ferric oxide (Fe_2O_3) in 0.2 ml of 0.15 M NaCl at weekly intervals. After the last instillation, the hamsters were randomly assigned to receive either 100, 1600, or 3300 (later reduced to 2400) µg retinyl acetate (RA) per week in divided intragastric doses. Hamsters in the 2400 µg RA group had a significantly higher incidence of respiratory tract tumors than those in the group given 100 µg RA per week. Liver vitamin A stores increased dramatically in the groups given 1600 and 2400 µg RA and corresponded to the administration of RA p.o. Serum vitamin A values were not consistently related to RA administration or to hepatic stores of vitamin A.

Male Syrian golden hamsters were fed a semisynthetic diet, given 12 weekly intratracheal instillations of 3 mg BP adherent to 3 mg Fe_2O_3, and then given either 100, 1600, or 2400 µg RA per week intragastrically in two divided doses. Half the animals in each group were housed conventionally; the other half were housed in laminar flow units.

Hepatic and serum vitamin A levels were markedly increased in hamsters given 1600 or 2400 µg RA per week compared to controls given 100 µg, which is adequate for growth and maintenance.

In hamsters housed conventionally, increased RA intake was associated with an increased incidence of benign respiratory tract tumors. In all groups of hamsters housed in laminar flow units there was a longer period to death with respiratory tract tumor than in conventionally-housed hamsters; increased RA intake was associated with a somewhat lower incidence of respiratory tract tumors. Laminar flow housing significantly reduced the incidence of respiratory tract infection in non-tumor-bearing hamsters. Squamous papillomas of the forestomach were significantly reduced in all groups of hamsters given high levels of RA regardless of housing.

Significance to Biomedical Research and the Program of the Institute: The optimal outcome of these studies will depend on how fully we can develop good animal models for the intelligent use of vitamin A or vitamin A analogs as anti-carcinogenic agents. These studies in turn will depend on a better understanding of the cellular and biochemical processes controlled by vitamin

A. The present contract is immediately concerned with obtaining further information about the interactions between vitamin A and BP-induced lung cancer in the hamster.

Proposed Course: To complete the long-term animal studies now in progress so that complete evaluation of the gross and microscopic pathology can be made.

Date Contract Initiated: June 18, 1969

Current Annual Level: $101,259

MINNESOTA, UNIVERSITY OF (NO1-CP-33364)

Title: Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract

Contractor's Project Director: Dr. Lee W. Wattenberg

Project Officer (NCI): Dr. Carl E. Smith

Objectives: It has been demonstrated that several types of antioxidants will inhibit pulmonary neoplasia resulting from administration of polycyclic hydrocarbons. Specific objectives relevant to these observations are (1) elucidation of the mechanism of this inhibition; (2) determination of the conditions under which inhibition occurs; and (3) to ascertain optimal methods for utilizing antioxidants and related compounds to inhibit pulmonary neoplasia. In a second part of the proposed work objectives are to determine the relationship between the activity and composition of the aryl hydrocarbon hydroxylase system and the binding of metabolites of polycyclic hydrocarbons to macromolecules of pulmonary structures, and, in addition, to attempt to relate the amount of binding to the development of pulmonary neoplasia.

Major Findings: Butylated hydroxyanisole (BHA) inhibits pulmonary carcinogenesis resulting from administration of polycyclic hydrocarbons as well as a variety of other chemical carcinogens. Studies of the effect of BHA on the metabolism of polycyclic hydrocarbons by the microsomal mixed function oxidase system have been carried out in female A/HeJ mice fed BHA under conditions which inhibit BP carcinogenesis. Alterations in the metabolism of benzo[a]pyrene (BP) by the microsomal mixed function oxidase system were found. Incubation of BP and DNA with microsomes from BHA-fed mice resulted in significantly less binding of BP metabolites to DNA than with control microsomes. There is approximately one-half the binding of BP metabolites to DNA in the presence of microsomes from BHA-fed mice as compared to controls. Since changes in the amount of BP metabolite binding to DNA could result from an altered cytochrome P-450, the ethyl isocyanide binding spectra at pH 7.4 was measured. The peak at 430 nm was the same in control and treated animals. However, the peak at 455 nm was lower in the microsomes from BHA-fed mice than from controls. This resulted in a ratio of the maxima at 455 nm:430 nm that was significantly different in BHA-fed mice as compared to the controls. These spectral studies indicate the presence of an altered cytochrome P-450 but its nature remains to be determined. A further difference in the microsomal metabolism of aromatic polycyclic hydrocarbons brought about by BHA

feeding is the response to alpha-naphthoflavone (ANF). If ANF is added to the microsomal reaction mixture, considerably less of this flavone is required to produce inhibition of AHH with microsomes from BHA-fed mice than from control microsomes. In addition to the qualitative changes in the cytochrome P-450, it was found that BHA feeding causes an increase in the amount of P-450 per unit weight of microsomal protein and per unit weight of liver. Thus, the work that has been carried out with BHA has shown that this compound causes altered properties of liver microsomes including a decreased binding of BP metabolites to DNA.

Significance to Biomedical Research and the Program of the Institute: Man now consumes a substantial amount of antioxidants. In animal models these compounds have been shown to have the capacity to inhibit chemical carcinogenesis. It is of importance to know the mechanism of this inhibition and to evaluate the present and potential role of antioxidants as inhibitors of the effect of chemical carcinogens in the environment on pulmonary neoplasia. Such information could be of importance in minimizing the impact of environmental carcinogens on pulmonary neoplasia in man.

Studies on the relationship between the activity and characteristics of the aryl hydrocarbon hydroxylase system and the development of pulmonary neoplasia comprise the second portion of the present project. The AHH system is subject to alteration in activity and characteristics by pure compounds as well as dietary constituents. It is of importance to determine how these alterations may affect the susceptibility of the host to neoplastic response from chemical carcinogens.

Proposed Course: The proposed course includes studies of the mechanism of antioxidant inhibition of polycyclic hydrocarbon carcinogen-induced pulmonary neoplasia. Accompanying animal studies will determine conditions under which neoplasia is inhibited, and the relationships between chemical structure and capacity of compounds to exert inhibitory effects. Investigations are also in progress to determine the relationship between activity and characteristics of the AHH system and the extent of macromolecular binding of carcinogen metabolites to macromolecules and, in addition, the relationship of these parameters to the occurrence of pulmonary neoplasia resulting from exposure to polycyclic hydrocarbon carcinogens.

Date Contract Initiated: June 23, 1973

Current Annual Level: $123,195

NEW YORK UNIVERSITY (NO1-CP-33260)

Title: Studies in Pulmonary Carcinogenesis

Contractor's Project Directors: Dr. Marvin Kuschner
Dr. Sidney Laskin

Project Officers (NCI): Dr. Carl Smith
Dr. David Kaufman

Objectives: The objectives of this contract have been the investigation of the mechanisms of pulmonary carcinogenesis and the examination of the carcinogenic potential of suspected materials. Its aims are to illuminate the process of malignant change as exemplified in the lung while, at the same time, providing immediate means for the prevention of lung cancer by the identification of hazardous materials. In particular, since it has become more and more evident that the induction of human lung cancer is multi-factorial in nature and dependent upon long-term, low-level exposures to combined agents, one objective of this contract is to evaluate this combined action in a wide variety of animal models using a variety of experimental techniques.

Major Findings: Animal models have been developed which duplicate human bronchogenic carcinoma. The production of such tumors has been achieved by a variety of techniques which include thread transfixion, pellet inplantation, direct intrabronchial instillation and, finally, inhalation. Dose-response relationships have been developed from the intrabronchial pellet technique with polycyclic hydrocarbons.

An extensive series of studies are being performed in this laboratory to study the effects of several components of air pollution. This laboratory was the first to produce lung cancer in rats with combined inhalation exposures to sulfur dioxide and benzo[a]pyrene (BP). Studies with combined carcinogen-irritant exposures were performed in an effort to reinforce the original results. Findings confirmed the original studies.

To define the progression of changes which result in cancer, an extensive dose-response study using hamsters and intratracheal intubations of methylcholanthrene was performed. Results showed an excellent dose-response relationship, a shortening of the time needed to develop cancer as dose increases, and cancer incidence approaching 100 percent at 24 intubations of 1 mg methylcholanthrene.

The hamster intubation technique has also provided a valuable model for carcinogen-irritant studies. Animals intubated with carcinogens were exposed by inhalation to sulfur dioxide atmospheres. Results have shown findings of adenocarcinoma in animals given 10 intubations of methylcholanthrene and lifetime exposures to sulfur dioxide. BP, which has shown negative results by intubation only, in combination with exposures to sulfur dioxide has shown both squamous cell carcinomas and adenocarcinomas in the lung.

Short-term and chronic inhalation studies with nitrogen dioxide, a component of air pollution and cigarette smoke, are complete. Although mucosal changes after exposures to 25 ppm appear to be minimal, endothelial damage has been reported with electron microscopy. Later results suggest a low incidence of panacinar emphysema, particularly in the hamster.

Studies have been initiated for combined exposures of rats and hamsters to nitrogen dioxide and BP. Early results, to date, have shown findings of two squamous cell carcinomas in rats exposed to nitrogen dioxide and BP for one hour per day.

Experiments with tobacco tar exposure by means of intrabronchial pellet inplants with large numbers of animals have been performed. Test materials in cholesterol carrier include BP alone, tobacco tar alone, and mixtures of the two. Results, to date, suggest specific effects of the acid washed tobacco tar. These include a squamous cell carcinoma of the trachea and a malignant tumor of undetermined type and origin in the lung.

Preliminary inhalation studies with croton oil have been completed. Studies involving carcinogen intubations with subsequent croton oil inhalation exposures are underway. Techniques employed involve the use of single or multiple intubations in hamsters with methylcholanthrene or BP followed by inhalation of croton oil. Early results show several squamous cell carcinomas in the hamsters intubated with BP followed by inhalation of croton oil.

Compounds of chromium have been demonstrated to be capable of producing bronchogenic carcinoma of the lung in experimental animals. The identification of calcium chromate as one of the major agents in the chromate problem has resulted in inhalation and clearance studies with this compound. The recently completed chronic study at 2 mg/cu m has produced carcinomas in rats and hamsters.

Studies are underway with current industrial chrome process materials provided by the industry. Since a survey of the industry and evaluation of experimental results implicates process residue as the major potential agent, a chronic inhalation study has been started with this material.

Two industrial plastic dusts were studied to determine their possible effects on workers in the industry. Reinforced polyester-fiberglass plant dust was studied utilizing the intubation technique and short-term inhalation exposures. Centrilobular emphysema and other suggestive changes of interest were noted. With polyurethane foam, animals were given 30 inhalation exposures to freshly generated foam dust atmospheres at two levels. Centrilobular emphysema and squamous cell carcinoma were seen in rats at both levels.

In view of the large population of construction workers involved in the use of such materials, chronic studies are being developed for the polyurethanes. Preliminary concentration and particle-size tests are in progress with current samples of the most commonly used polyurethanes. For these studies, a newly designed dust feed system has been developed. Additionally, a new sampling instrument for particle-size evaluation has also been developed.

Inhalation studies were completed with two chlorinated ethers used widely as industrial reaction intermediates. Both compounds proved to be carcinogens. The bis(chloromethyl)ether showed extremely high incidences of carcinoma at 0.1 ppm. The extremely low levels and the magnitude of the carcinoma response with bis(chloromethyl)ether suggested a very serious new industrial hazard. This has been reinforced by the publication of several reports documenting human cases in exposed workers. These compounds are now listed in the OSHA carcinogen regulations. Recently, preliminary studies in this laboratory suggest that hydrochloric acid and formaldehyde, at levels approximating industrial exposures, can produce bis(chloromethyl)ether. The widespread use of such materials indicates the need for animal inhalation studies.

Results with chloromethyl methyl ether and bis(chloromethyl)ether have prompted examination of a variety of other alkylating agents of industrial importance. Preliminary studies have been completed with the chlorinated ether dimethylcarbamyl chloride and a chronic inhalation study is underway. Preliminary studies have begun on epichlorohydrin.

Significance to Biomedical Research and the Program of the Institute: This contract represents a component of a broad effort to develop bioassay techniques for studies on lung cancer. It is also important for its study of potential hazards of materials which are in use industrially and may be found in the environment.

Proposed Course: To extend previous studies and develop inhalation studies with other types of suspected materials.

Date Contract Initiated: June 29, 1966

Current Annual Level: $459,555

ST. MARY'S HOSPITAL (NO1-CP-23233)

Title: Morphogenesis of Human Lung Cancer

Contractor's Project Director: Dr. Geno Saccomanno

Project Officer (NCI): Dr. Carl E. Smith

Objective: To learn more about the morphogenesis of human lung cancer by sputum cytological analysis with the correlation of cytology and histology of lung cancer.

Major Findings: In 1974, 2265 sputum samples from uranium miners were collected, slides prepared, reported, and filed. Nine new cases of carcinoma have been identified. Twelve cancer of the lung deaths occurred among the uranium miner population. Lungs from 14 autopsies and 2 surgeries were mailed to Dr. Oscar Auerbach, and these will be studied to collaborate cytological findings.

Significance to Biomedical Research and the Program of the Institute: These studies continue to contribute new knowledge on how cancer of the lung develops cytologically in time, and more and more information is accumulating to assess the average length of time that these lesions stay in the in situ state. These studies also indicate that cancer of the lung in uranium miners develops essentially the same in non-miners, and this has a very important significance to the population at large who develop this malignancy. In this light, also, a protocol for the comparisons of the uranium miners' lungs with the lungs of non-miners has been designed.

Proposed Course: Activities under this contract will be completed during the present fiscal year. A large amount of data has been accumulated requiring study before a future course for the efforts is known.

Date Contract Initiated: May 1, 1972

Current Annual Level: $137,000

SOUTHERN RESEARCH INSTITUTE (NO1-CP-22064)

Title: Organ Culture and Tissue Culture Bioassay of Vitamin A Analogs

Contractor's Project Directors: Dr. Lee Wilkoff
Dr. Donald Hill

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The objectives of this contract are (1) to develop adequate bioassays to evaluate the biological activity of new vitamin A analogs, as measured by control of epithelial differentiation; (2) to perform bioassays on selected analogs; (3) to develop new quantitative assays for vitamin A activity in organ culture or tissue culture; and (4) to determine the effects of vitamin A compounds on the metabolism of carcinogens.

Major Findings: A bioassay has been developed to evaluate vitamin A analogs for activity in the control of epithelial differentiation. With organ cultures of 13-day chick embryo skin, 17 vitamin A compounds have been tested as inhibitors of keratinization and as inducers of mucous metaplasia. Ro8-7699, a cyclopentene analog of retinoic acid (RA), was 10-fold more effective than RA in these tests. Analogs with activity approximately equivalent to RA were the trimethylmethoxyphenyl analog of RA, α-RA, 13-cis-RA, RA methylester, RA ethylester, and RA ethylamide.

In extracts of chick embryo skin, a protein which binds RA and its biologically-active analogs having a free carboxyl group was found. Analogs that were most active in the organ culture assay bound most readily to the protein which may be involved in the biological activity of the compounds.

An organ culture system for mouse prostatic tissue has also been established. Explants cultured in the presence of 3-methylcholanthrene displayed increased proliferative activity, which was inhibited by RA.

Some vitamin A compounds inhibited an oxidative reaction catalyzed by a constitutive enzyme in lung microsomes and leading to irreversible binding of benzo[a]pyrene (BP) to macromolecules. Since this reaction was also inhibited by butylated hydroxytoluene, an anticancer agent, the reaction may be involved in carcinogenesis by BP.

Significance to Biomedical Research and the Program of the Institute: Vitamin A is required for the normal differentiation and growth of the epithelium in the following organs, all of which are major sites of primary cancer in man: bronchi and trachea, colon, stomach, uterus, kidney and bladder, testis, and skin. The role of vitamin A in controlling normal differentiation and growth in tracheobronchial and intestinal epithelium is currently under investigation in the lung cancer program of the NCI.

In addition to controlling the differentiation of many types of normal epithelium, vitamin A is also able to alter the process of chemical carcinogenesis in several different epithelia and to block the effects of several different carcinogens. Thus, vitamin A can block the carcinogenic effects of polycyclic hydrocarbons on tracheobronchial, gastric, and uterine (cervical) epithelium. In some of these studies, it has been shown that the effect of vitamin A may not be due to alterations of the immediate metabolism of the carcinogen, since vitamin A is able to inhibit carcinogenesis even when administered several weeks after the carcinogen. Studies are currently in progress in the lung cancer program to elucidate the role of vitamin A in blocking epithelial carcinogenesis. Presumably this effect is directly related to the biochemical and cellular role of vitamin A in determining normal differentiation in these tissues, but the proof of this is not yet at hand.

The ultimate implications of these studies on the inhibition of epithelial carcinogenesis are very important. If vitamin A is indeed functioning as a differentiation hormone, it should be possible, with the skill and cooperation of a group of organic chemists, to design and synthesize new analogs of vitamin A having even greater potency than vitamin A on differentiation of epithelium. The precedents in steroid chemistry for this approach to vitamin A are impressive; presently there are many synthetic steroid analogs that are more potent (and often more specific and less toxic) than the naturally occurring estrogens, progestational agents, androgens, or adrenocortical steroids. If this type of approach could be applied to the hormonal action of vitamin A, the potential for inhibiting carcinogenesis in many types of epithelia would be greatly increased.

Proposed Course: Continue the development of bioassay methods using organ culture systems to evaluate the biological activity of vitamin A analogs. Devise new methods, using morphological, histochemical, and biochemical end points for quantitative bioassay of vitamin A and vitamin A analogs. Measure and evaluate the inhibition by vitamin A compounds and analogs of microsomal oxidases of lung and other epithelial tissues that are responsible for metabolism of polycyclic hydrocarbons. Determine the tissue distribution and biochemical characteristics of retinoic acid-binding protein, and attempt the development of biochemical screen for vitamin A activity using this protein.

Date Contract Initiated: December 10, 1972

Current Annual Level: $254,400

STANFORD UNIVERSITY SCHOOL OF MEDICINE (NO1-CP-43295)

Title: Studies on Oat Cell Carcinoma of the Lung

Contractor's Project Director: Dr. Klaus G. Bensch

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: Development of a reliable means for identification of the bronchial counterpart of the intestinal argentaffine (Kulchitsky) cells (K-cells),

their distribution in the human lung, their adaptation to organ and cell culture, and elucidation of factors in their neoplastic transformation.

Major Findings: A continuing effort is being made to develop a (specific) reproducible stain for the identification of bronchial K-cells; adaptation of the Grimelius silver nitrate technique has resulted in the demonstration of these cells in animal, but not human, lung tissues. Survival of K-cells in organ cultures of human bronchi up to four weeks occurred, but it was accompanied by progressive loss of their neurosecretory-type granules. Similarly, four human pulmonary carcinoid tumors in tissue culture lost most of their complement of granules over a period of one to two months. Analyses for the presence of several key enzymes of the catechol and indole metabolism of oat cell tumors, pulmonary carcinoid tumors and tissue and organ cultures of both types of neoplasms are being continued. Unfortunately, cell lines retaining the ability to produce neurosecretory-type granules and assumed specific products have not been isolated. Such cell lines would undoubtedly be of immense help in this study.

Significance to Biomedical Research and the Program of the Institute: Oat cell carcinomas of the lung arise from a specific type of cell of the human respiratory tract. Although these cells appear to be present as a relatively small proportion of the population of the bronchial and bronchiolar lining, the incidence of this type of tumor is very high in males. Our studies should shed light on the distribution of these cells in human bronchi, and experiments with in vitro cultures of these cells are aimed at identification of factors, or of mechanisms, active in the neoplastic transformation of these cells.

Proposed Course: Development of means for ready identification of K-cells, including the use of fluorescent antibody techniques; distribution, replication and growth pattern of these cells; isolation and identification of specific secretory products; uptake by these cells of compounds known to be associated with high incidence of oat cell tumors and their effect on cell growth and replication.

Date Contract Initiated: September 4, 1974

Current Annual Level: $87,862

STATE UNIVERSITY OF NEW YORK (At Stony Brook) (NO1-CP-33361)

Title: Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi

Contractor's Project Director: Dr. Bernard Lane

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: (1) to determine the methodology for testing for biologic (in vivo) malignancy of tracheal epithelium transformed in organ culture; (2) to determine the optimal conditions for the organ culture of tracheal rings; (3) to observe the biology of tracheal ring cultures; (4) to determine the

effects of exposing tracheal rings to the carcinogen benzo[a]pyrene (BP) in vitro; and (5) to monitor cultures exposed in vitro for malignant transformation.

Major Findings: (1) Work during the previous contract period established that Wistar Lewis rats were suitable as both donor and host for studies involving implantation of carcinogen-treated organ cultures of trachea into intact animals to test for malignant potential of the carcinogen-altered tissue. Careful study of the process of rejection of cultures by outbred hosts has now been performed to eliminate host response to incompatible tissue. This is the first step in determining whether immune response of compatible hosts to carcinogen-exposed tissues can serve as a sensitive indicator of malignant transformation. Lymphocyte-mediated epithelial cell injury was seen to occur 15-21 days after implantation of Sprague-Dawley derived cultures into Sprague-Dawley host animals. The injury followed a period of lymphocyte infiltration of the epithelium during which there was blast transformation in the implant but no injury to the epithelial cells of the implanted cultures.

(2) Methods for large-scale long-term culture of rat tracheal tissues were developed during the current contract period. These were based upon previous work which established that McCoy's 5a (modified) medium buffered to pH 6.8-7.3 with sodium bicarbonate and maintained in an atmosphere of 5% CO_2 with air will support growth. Using a mechanical slicing device, cultures are prepared in batches of 300-500 and grown under these conditions for periods of months. The cultures are uniform in appearance and in the ability to respond to various stimuli by cellular repair and by mitotic activity. Studies of epithelial growth and maintenance of differentiation in CMRL 1066, Parker's 199 and Dulbecco's modification of Eagle's MEM demonstrated that these alternative culture media offer no advantage over McCoy's 5a(modified) medium. A study of the role of serum in epithelization of the free floating culture, using light, transmission electron and scanning electron microscopy, established the basis for observed differential patterns of the appearance of ciliated cells and dividing cells on the surfaces of the culture. Serum was shown to facilitate migration of the epithelial cells as a continuous flattened sheet.

(3) A series of protocols for exposure of the cultures to carcinogens were carried out. Continuous exposure to BP in the culture medium was found to result in shortening of the life of the culture in rough proportion to the concentration of this carcinogen. A maximal effect, consisting of hyperplasia, after two weeks of exposure to 2 µgm/ml BP gives way to atrophy if continued for several weeks longer. To avoid the apparent masking of carcinogenicity by cytotoxicity of the agent, two modified exposure regimes were evaluated. When 30-hour exposure periods were alternated with 72- to 138-hour periods in medium lacking the carcinogen, cell death occurred as observed with continuous exposure. However, cultures exposed continuously for two weeks to 2 µgm/ml and then removed to carcinogen-free medium developed areas of focal hyperplasia with high level of thymidine incorporation. These focal hyperplasia, which may be equivalent to modular hyperplasias in livers of animals treated with carcinogens, are being studied for persistence, response to promoters, reversibility with vitamin A and growth characteristics in a host.

(4) The carcinogen-treated cultures are being studied to identify markers of malignant transformation which can be recognized before diagnostic histologic changes appear. Work has begun on growing, in monolayer culture, cells derived from normal epithelium and carcinogen-altered epithelium. Dissection of cells manually or by treatment with collagenase, results in release of single cells or small groups of cells which attach to the culture dish and maintain viability for a period of a week. Propagation of these cells has not yet been achieved but experiments measuring the effects of growth factors on the cells are underway. In addition, several cytochemical approaches are in progress addressed to the problem of early recognition of malignant transformation. Electron microscope methods, using osmiophilic tetra zolium salts, lead capture of phosphates liberated by esterases and specific labelled antisera against enzymes are being applied to normal tracheal epithelium. Cell cycle data on stimulated normal cells have been obtained, using the technique of double-labelling with radioactive thymidine to delineate a cycle time of 28 hours. The values for carcinogen-altered populations will be compared.

Significance to Biomedical Research and the Program of the Institute: The tracheobronchial epithelium gives rise to more fatal human tumors than any other tissue. Yet experimental access to many parameters of carcinogenesis under controlled conditions, including dependable tests of malignant transformation, has been limited. Long-term organ culture of tracheobronchial epithelium constitutes a model which preserves the biologic organization of the tissue while permitting control of almost all variables affecting population kinetics and cellular differentiation, stages in carcinogenesis and modification of carcinogen effects by other agents. This contract, and a companion contract to it, is directly concerned with the problems of determination of causes of lung cancer, with the early diagnosis of lung cancer lesions, and with effective prevention of lung cancer.

Proposed Course: The proposed course is to transplant rings which have been transformed during culture in media containing BP, to monitor the hosts for metastasis and to examine recovered transplanted rings and the surrounding tissue for invasive growth. At the same time, methods for monitoring early transformation in vitro including ultrastructural changes, binding of carcinogen in the target cell, karyotype differences, changes in the phases and timing of cell cycles, the appearance of new antigens, deletional mutations observed by histochemical, biochemical or immunological techniques, and changes in sensitivity to mitogens are to be studied. A technique for culturing the population of epithelial cells which we can now isolate will be sought in order to facilitate some of these studies especially those involving biochemical changes. The ability to stimulate proliferation of carcinogen-induced cell populations with promoters and to inhibit their growth with vitamin A will be studied.

Date Contract Initiated: June 25, 1973

Current Annual Level: $129,133

STOCKHOLM, UNIVERSITY OF (NO1-CP-33363)

Title: Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract

Contractor's Project Director: Dr. Lars Ernster

Project Officers (NCI): Dr. Carl E. Smith
Dr. David G. Kaufman

Objectives: The objective of this contract is to define the relationship between the enzyme system aryl hydrocarbon monooxygenase and pulmonary neoplasia. Specific aims are (1) the determination of the distribution of substrates and products of aryl hydrocarbon monooxygenase in pulmonary tissue; (2) subcellular fractionation of lung cells and subcellular localization of aryl hydrocarbon monooxygenase activity; (3) employment or development of a highly sensitive assay for aryl hydrocarbon monooxygenase, including studies of tissue disruption to optimize enzymatic activity accessible to assay; (4) dissociation of the cells of the lung and isolation of individual cell types; (5) characterization of the lung aryl hydrocarbon monooxygenase system, including identification of the metabolites of polycyclic hydrocarbons in pulmonary tissue; (6) isolation and characterization of lung cytochrome P450; (7) characterization of pulmonary epoxide hydrase and glutathione transferase activities; (8) studies on polycyclic hydrocarbon metabolism in isolated intact hepatocytes; and (9) initial studies of this metabolism in human lung.

Major Findings: (1) A sensitive and convenient radioactive assay for benzpyrene monooxygenase has been developed based on the use of tritium-labeled substrate and separation of substrate from products by a simple extraction procedure. (2) An investigation has been made of the applicability of a direct fluorometric assay for benzpyrene monooxygenase to rat lung tissue. (3) The new radioactive assay has been employed in studies of various aspects --including kinetics, inhibition, effects of inducers, nature of the products-- of benzpyrene metabolism by isolated intact rat hepatocytes. (4) The fluorometric assay has been used to investigate the kinetics and inducibility of benzpyrene monooxygenase in rat lung. (5) A method has been developed for removing hemoglobin from lung microsomes by chromatography on Sepharose. (6) The spectral properties of cytochrome P450 have been investigated in lung microsomes from which hemoglobin has been removed. (7) Lung cytochrome P450 has also been isolated and the properties of the purified form studied. (8) Subfractionation studies on lung microsomes have been carried out involving selective aggregation of fragments of the endoplasmic reticulum with cesium ions.

Significance to Biomedical Research and the Program of the Institute: Many of the foreign compounds which enter the human body do so through the lungs. Among these are the polycyclic hydrocarbons which may be a major cause of human cancer. The principal pathway for metabolism of the polycyclic hydrocarbons in mammalian tissues is the inducible enzyme system aryl hydrocarbon hydroxylase. This contract and others in the program are directed towards a detailed understanding of the nature and function of this enzyme system and its relationship to carcinogenesis in the respiratory tract.

Proposed Course: (1) To continue studies of benzpyrene metabolism in isolated intact rat liver cells with special emphasis on the products formed under various conditions (e.g., lowered intracellular levels of glutathione); (2) to investigate the further metabolism of 3-hydroxybenzpyrene by rat lung microsomes; (3) to characterize the induction kinetics and subcellular localization of epoxide hydrase (using styrene oxide as substrate) in rat liver and lung; (4) to determine which of the five forms of glutathione transferase in rat liver is the glutathione S-arene oxide transferase of interest in polycyclic hydrocarbon metabolism and characterize this enzyme in rat liver and lung; and (5) to isolate different cell types from rat lung to ascertain the cellular distribution of the different enzymes of polycyclic hydrocarbon metabolism.

Date Contract Initiated: June 25, 1973

Current Annual Level: $179,848

TEXAS, UNIVERSITY OF (Southwestern Medical School at Dallas) (NO1-CP-33362)

Title: Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract

Contractor's Project Director: Dr. Ronald Estabrook

Project Officer (NCI): Dr. Carl E. Smith

Objective: The objective of this contract is to define the relation between the enzyme system, aryl hydrocarbon hydroxylase, and pulmonary neoplasia. Specific aims are (1) separation and isolation of the microsomal fraction of lung tissue from unexposed and polycyclic hydrocarbon-induced animals; (2) physicochemical and enzymatic characterization of the isolated cell fractions, including the cytochrome P-450 and P-448 components, and the isolation of these components; and (3) complementary studies on the isolation of NADPH-cytochrome P-450 reductase from microsomes of lung tissue.

Major Findings: The research approach taken in this study has been to contrast the lung microsomal arylhydrocarbon hydroxylases to those of liver. Using several chemical and immunochemical inhibitors, the normal and carcinogen-induced cytochrome P-450-dependent monooxygenases have been investigated to detect any differences between the enzyme systems of lung and liver. The basic electron transport system associated with cytochrome P-450 is very similar in both organs. No differences are seen immunochemically between the flavo-protein reductases of cytochrome P-450 or cytochrome b_5 in liver or lung microsomal fractions. Experiments using chemical inhibitors of benzo[a]pyrene hydroxylase or biphenyl hydroxylase (a model arylhydrocarbon hydroxylase) show that the lung contains a constitutive aromatic hydroxylase which is quite active but does not hydroxylate benzo[a]pyrene well. A 3-methylcholanthrene inducible cytochrome P-450-dependent aromatic hydroxylase exists which appears to hydroxylate benzo[a]pyrene but does not hydroxylate biphenyl. This study indicates that there exist two types of cytochrome P-450-dependent aromatic hydroxylases in lung; only the inducible hydroxylase appears to be capable of metabolizing benzo[a]pyrene.

Significance to Biomedical Research and the Program of the Institute: Polycyclic hydrocarbons and their metabolites are recognized as potential carcinogenic agents. A major route of introduction of polycyclic hydrocarbons into the human is through the respiratory tract. The primary mechanism of metabolism of polycyclic hydrocarbons involves oxygen activation by a unique hemoprotein-containing electron transport system associated with the endoplasmic reticulum of many tissues. This contract and others in the program are directed toward a detailed understanding of this enzymatic system functional in the oxidative metabolism of polycyclic hydrocarbons and its role in respiratory tract carcinogenesis.

Proposed Course: The metabolism of aromatic hydrocarbons in lung and the enzymology of benzo[a]pyrene hydroxylase will continue using techniques capable of monitoring total metabolite formation.

Date Contract Initiated: June 20, 1973

Current Annual Level: $35,069

VERMONT, UNIVERSITY OF (NO1-CP-33360)

Title: Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi

Contractor's Project Director: Dr. John E. Craighead

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: This project has five basic objectives: (1) the development of organ culture systems which permit viability and differentiation of hamster tracheal epithelium for extended periods of time; (2) the development of techniques for the application of carcinogens quantitatively to respiratory tract epithelium in organ culture; (3) the development of techniques for the transplantation of organ culture tissue into susceptible host to document in vivo neoplastic transformation due to carcinogen exposure in vitro; (4) an assessment of the carcinogenic properties of selected carcinogens in differentiated respiratory epithelium; (5) the detection of possible type-C virus activation in animal tissue treated with carcinogen in vitro.

Major Findings: Various chemically-defined media and the effects of serum, insulin, vitamin A, and adrenal corticosteroids on long-term cultures (i.e., 8-24 weeks in vitro) of respiratory epithelium have been systematically explored. Tissues were evaluated at weekly intervals by histology, autoradiography and assay for acid phosphatase release. Proliferative epithelial changes were observed in organ cultures maintained in "complex" media containing serum whereas use of these media in the absence of serum of vitamin A produced varying degrees of squamous metaplasia and keratinization. Addition of hydrocortisone enhanced keratinization and release of acid phosphatase in these systems.

Glass and Lycra fibers have been dipped in various concentrations of radiolabeled 3-methylcholanthrene and benzo[a]pyrene in acetone and applied (after evaporation of the acetone) to the epithelial surface of the organ cultures.

This technique permits us to vary the amount of carcinogen applied to the epithelium and allows a systematic evaluation of epithelial changes at a defined site over a range of time periods. Detailed studies have shown that the concentration of hydrocarbon on the fiber can be accurately regulated. Observations indicate that epithelial cells exhibit cytologic alterations and changes in ^{3}H-thymidine uptake at sites of fiber application after brief periods of exposure. Proliferation of the affected mucosa and loss of orientation of epithelial cells is noted.

Tracheal rings maintained in organ culture for up to six months have been implanted into subcutaneous sites and into cheek pouches of syngeneic animals for periods of as long as 52 weeks. As of this date, more than 500 organ cultures, including controls, have been implanted into more than 50 weanling hamsters. At present approximately 30 percent of these animals have palpable subcutaneous implants which appeared at roughly eight months after implantation. Histological examination of several of these implants upon sacrifice or death of the host revealed proliferation of a migratory epithelium resembling mucin-secreting cells. Metastases to other organs had not occurred.

Significance to Biomedical Research and the Program of the Institute: The association of bronchogenic carcinoma with cigarette use is well established on epidemiologic grounds. Although a number of cigarette smoke and tar constituents have been identified as probable carcinogens or co-carcinogens, their mode of action upon the tracheal-bronchial tree remains undefined. A number of studies have explored the effects of polycyclic hydrocarbons on epithelial cells and fibroblasts in monolayer culture; however, since cells in monolayer are relatively undifferentiated, this approach does not provide definitive information on the carcinogenic effects of inhalants on the differentiated epithelial cells of the respiratory tract. Maintaining the differentiated respiratory epithelium in organ culture for extended periods of time allows a systematic evaluation of carcinogenic agents and their mechanism of interaction with the differentiated respiratory epithelial cell.

Proposed Course: To conduct further studies in which hydrocarbons bound to particulates (carbon, asbestos, Fe_2O_3) are precipitated onto epithelial surfaces of explants. Electron microscopy of selected specimens has shown that the particles are taken up by the epithelial cells and that these cells are morphologically altered. The carcinogenic potential of these tissues will be assessed by transplantation into animal hosts.

Date Contract Initiated: June 28, 1973

Current Annual Level: $114,215

VETERANS ADMINISTRATION HOSPITAL (Tampa, Florida) (YO1-CP-55625)

Title: Autoradiographic Study of the Cellular Response of the Respiratory Tract in Chemical Carcinogenesis

Contractor' Project Director: Dr. Hollis G. Boren

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: To determine proliferative cellular response of segments of the respiratory tract following exposure to respiratory carcinogens and particulates, using primarily the hamster intratracheal instillation model. These measurements are correlated with the histopathological response to carcinogens and particulates. Radioactive carcinogens, anti-carcinogens and other compounds are localized in the respiratory tract and their amounts are determined by quantitative light microscopic autoradiography. Serial sacrifice studies are performed to define the morphogenesis of squamous cell carcinoma of the lung, as well as the determination of which preneoplastic lesions are modified by vitamin A compounds.

Major Findings: Double labeling with ^{3}H-thymidine followed later by ^{14}C-thymidine showed that the duration of the S-phase of DNA synthesis for basal cells was 6.8 ± 1.3 hours and the duration of the post synthesis gap (G_2) was 9 hours. For mucous cells the duration of G_2 was less than 9 hours. The exact duration of the S-phase for mucous cell is being determined by using short periods between double labeling. There were no significant numbers of ciliated cells that labeled with thymidine.

By the use of long exposure times in vivo binding of small amounts of ^{3}H-benzo [a]pyrene over a period of 2 hours to 28 days was determined in male hamsters eating laboratory chow. Binding persisted for the entire 28-day period. All cell types were labeled. There was no difference between the binding over nucleus as compared to that over cytoplasm. These findings were compared to the binding of ^{3}H-benzo[a]pyrene in vitamin A-deficient hamsters. There was significantly greater binding of carcinogen in both non-metaplastic respiratory epithelium and in areas of squamous metaplasia.

The relationship between thymidine incorporation as determined by quantitative autoradiography and sham treatment, exposure to ferric oxide, and exposure to benzo[a]pyrene and ferric oxide was found to be related to the carcinogen but not to the ferric oxide.

The long-term histogenesis experiment has shown two squamous cell tumors, one in a hamster on 675 micrograms retinyl acetate twice a week and another in an animal on 224 micrograms of retinyl acetate twice a week. Observations through 37 weeks of the experiment have shown a variation between the numbers of labeled cells but no change in the duration of the S phase. Continuous labeling has shown that ciliated cells are labeled with thymidine after seven days, giving an indication of the time for ciliary differentiation from basal or mucous cells.

Significance to Biomedical Research and the Program of the Institute: This contract is an integral part of an interlocking group of contracts designed to define the role of carcinogens and the physico-chemical factors (e.g., particulates) required for respiratory carcinogenesis. The other studies, including long-term tests, have been designed so that they are closely related to the present one with maximum interaction but no overlap of activity, in an effort to define the key role of vehicles and particulates in the process of respiratory carcinogenesis.

Proposed Course: Measurement of cell kinetics using double labeling with ^{3}H- and ^{14}C-thymidine, continuous labeling, and colchicine will be continued. The time interval between the two different thymidine-labeled compounds will be varied to determine the duration of S an G_2 in mucous cells. The effect of carcinogens and the vitamin A status of animals upon the duration of S will be determined. Experiments on binding of benzo[a]pyrene will be extended to determine the effect of multiple doses of carcinogen, of administered vitamin A and of cell proliferation upon immediate and long-term binding of carcinogen. Binding after exposure to non-carcinogenic stimuli (SO_2) will be compared to that of carcinogens.

The effects of both carcinogen and vitamin A upon incorporation of thymidine is in progress with the histogenesis experiment. The histogenesis experiment will be largely completed this coming year. The effect of vitamin A state response will be determined by a vitamin A dose response study.

Date Contract Initiated: June 2, 1972

Current Annual Level: $127,485

7. PUBLICATIONS OF THE CARCINOGENESIS PROGRAM

A. INTRAMURAL PROGRAM[1]

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A35. DiPaolo, J.A.: Karyological instability of neoplastic somatic cells. In Vitro (In Press).

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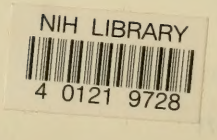
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